The Metabolism of Phosphorus, Copper and Molybdenum and Their Interrelationships In the Animal Organism

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INTRODUCTION

Naturally occurring nutritional abnormalities due to mineral deficiencies and excesses have been reported in Florida, in other states, and in various parts of the world. Extensive investigations have been made of the diseases as they affect livestock and laboratory animals. In the study of copper deficiency in cattle, the existance of a relationship between molybdenum, phosphorus, and copper has been noted, and the influence of these elements upon each other in body metabolism and their relationship to the general health, breeding, reproduction, and growth of livestock has been the object of this investigation.

In many soils of Florida, copper is borderline in regard to the nutritional requirement. In the muck or peat soils of the Everglades and the smaller peat deposits throughout the state, copper fertilization is necessary to produce truck crops (3, 69). Cattle grazed on the pastures of these areas have frequently shown symptoms of abnormal mineral-copper metabolism. The analysis of some of these pastures have given copper values ranging between two and one-half and four parts per million, whereas other non-peat borderline areas have been reported with as high as seven parts per million of copper. The molybdenum content of some forage from these muck and peat areas has been far in excess of the levels found in normal forage (34). Cattle growers have found that it has been necessary to use copper supplements on these copper deficient or high molybdenum soils as top dressing for pastures, in mineral mixtures, or as drenches in order to use more efficiently areas in which cattle production has almost been an impossibility.

The symptoms which have, in general, resulted from continued grazing of cattle on these pastures, without regard to corrective measures, include severe diarrhea, low hemoglobin, emaciation, loss of weight, rough skin, fading of the haircoat, coarseness of the haircoat, tendency for sores to heal slowly, swelling of the ends of the long bones of the legs of calves, beading of the ribs, fragile bones and ribs, swelling of the joints, and generally, a picture of the early stage of rickets (34). In the past a number of observations have indicated that copper deficiency may result in bone changes and abnormalities. There appears to be a definite retardation of normal phosphorus deposition in the bones of animals grazed on some of the affected pastures. The cells of the periosteum, as a result of inadequate phosphorus deposition, regress so that the union with the bone matrix is destroyed and an actual separation may result. Davis (34) has also indicated that there is an erosion of the joints which develops into an arthritic-like condition. This condition is generally associated with a low phosphorus intake and results in a stiffness of the legs of the animal. This is accompanied by inflammation and pain. Seemingly a high molybdenum content of the ration accentuates a copper deficiency, which is in turn apparently related to a possible phosphorus deficiency. Copper deficiency also results in poor breeding efficiency with small calf crop yield. Another effect has been the death of some animals, apparently from heart failure, as indicated by the suddenness of death and the lack of gross clinical symptoms.

In the present investigation, rats have been used to study the effect of copper and molybdenum, in varying amounts in the ration, upon

growth, hemoglobin values, pigmentation of the haircoat, and the production of bone abnormalities, and their effect on reproduction. The deposition of copper and molybdenum in the liver and molybdenum in the bone has been investigated. The distribution of copper, molybdenum and phosphorus in selected tissues and the excretion of each element administered alone, in combination with the others, and under specific feeding trial conditions, have been studied in the bovine and laboratory animals by the use of the radioactive isotopes of copper, molybdenum and phosphorus.

REVIEW OF LITERATURE

Although the essentiality of copper in nutrition has been recognized for a relatively short time, the discussion of its occurrence in nature can be traced as far back as 1817 to the work of Meissner (130), who established the fact that copper is actually a constituent of plants. In 1847 Harless (81) detected copper in the marine animals, Mytilus and Helix pomatia, and demonstrated that it did not exist as a free salt, but rather in combination with blood proteins. In 1920 Rose and Bodansky (159) reported that copper is a normal and possibly an essential constituent of marine fish tissue. A review of the early historical development of the biological significance of copper has been presented by Elvehjem (56).

While the earlier workers considered the presences of copper in animal tissue to be of no significant importance except in the lower animals, where it occurred as hemocyanin, McHargue (120) in 1925, stated that copper is a necessary constituent of the blood of all animal life and probably performed important functions in the absorption and transfer of oxygen in the respiratory process. The high accumulation of copper in the fetuses of mammals was accepted as strong evidence that copper has many important functions in the development of the embryo and in early growth after birth. This has been confirmed by other investigators (20, 30, 111, 117, 143, 145, 160). In humans it has been observed that the maximum content of copper in the liver is found at birth. There is a rapid decline of copper after the second month of life (20).

Other workers (30, 98, 101, 121, 138, 152, 188) have established

the necessity of copper for the mobilization of iron from the tissues and for the utilization of iron in the formation of hemoglobin. The action of copper in iron metabolism and its role in hemoglobin regeneration has been given extensive investigation. McHargue et al (121) in 1928 were among the first to show that young anemic rats required copper for the formation of hemoglobin. In 1928 Hart et al (82) found that copper in minute amounts is capable of supplementing ferric chloride, which in itself was ineffective in the regeneration of hemoglobin. These results were interpreted as indicating the necessity of copper for the effective utilization of iron for hemoglobin formation. Elvehjem and Hart (57) in 1929 presented a further demonstration of the supplementing action of copper to iron on the regeneration of hemoglobin. Cunningham (30) in 1931 confirmed the effect of copper in promoting the utilization of iron in hemoglobin formation. In the same year Drabkin and co-workers (46) questioned the specificity of copper in hemoglobin synthesis. The essentiality of copper for hemoglobin formation has been confirmed by other investigators (30, 55, 58, 60, 82, 98, 100, 101, 102, 109, 121, 132, 138, 152, 188). A comprehensive review of the role of copper in blood formation was presented by Schultze (170) in 1940. Evidence is presented that copper is not a constituent of the hemoglobin molecule, but that it is required for the production of hemoglobin.

The successful treatment of hypochromic anemia (133), hemorrhagic anemia in animals on milk diets (92, 193), hepatic cirrhosis (167), and various nutritional anemias (121, 171, 176, 188, 189) has been reported by many investigators. The production of anemia in rats by the use of a

milk diet is a conventional practice. It has been reported that copper sulfate stimulated the production of new erythrocytes in young rats made anemic on a milk diet, but had no effect on hemoglobin (149). The administration of both ferrous sulfate and copper sulfate resulted in an increased level of hemoglobin and the formation of erythrocytes (150). Titus and Hughes (183), earlier investigators, found that nutritional anemia could not be produced in animals on a milk diet supplemented with iron and copper. In experiments with rats, it has been observed that copper causes a temporary rise in hemoglobin, but does not cure nutritional anemia (110). Seagard (174) has concluded in a survey of the literature on the action of copper in nutritional anemia that it is necessary for hemoglobin formation and is a specific catalytic agent in its formation. The effects of copper on anemia induced by bleeding has also been investigated (38, 55, 107, 158).

The relation of other minerals and copper in metabolism has received some attention. Copper apparently increases iron absorption under certain conditions (105). Elvehjem (56) in 1935 reviewed the early literature concerning the role of copper in the treatment of anemia. Low levels of iron or copper will cause a decrease in food intake, growth and hemoglobin of rats (94). When the manganese intake of rats is greater than 100 micrograms daily, there is a decreased copper storage (148). The absorption of copper from the alimentary tract is influenced by the gastric acidity and the calcium content of the diet (184).

The distribution of copper among the different constituents of normal blood has been reported by many investigators. Guillemet (77)

found in beef blood that the whole blood contained 0.088 milligrams, plasma 0.140 milligrams, and washed red cells 0.025 milligrams, expressed in milligrams per 100 milliliters. The copper content of other species has also been reported in the literature (1, 77). Kehoe et al (99) have reported that blood copper is almost evenly divided between the blood plasma and the formed elements.

The number of radioactive copper studies so far reported in the literature are few. Schubert et al (168) in a tracer study with radioactive copper, produced by irradiation of copper with deuterons, found that when the copper was injected intravenously into two dogs as a copper sulfate-saline solution, it was slowly absorbed by the blood cells and retained for a considerable time. After 10.5 hours the greatest accumulation of radioactive copper was found in the liver and decreasing amounts in the kidney, lung, heart, and pancreas. Yoshikawa and his co-workers (196) reported that radioactive copper would appear in the plasma within several hours after administration, but was not present in the red blood cells until the peak of concentration in the plasma had been passed. Schultze and Simmons (172) reported the greatest concentration of radioactive copper in the liver, kidney, and the bone marrow. The presence of the high concentration in the bone marrow is thought to be indicative of the connection between copper and red cell maturation.

The copper content of many foodstuffs is reported in the literature (30, 74, 93, 101, 106, 109, 110, 115, 154). The presence of copper has been reported in human, goat, and cow milk (41). Analysis of the tissues of various animals has also been extensively reported. Lindow

et al (115) have made an extensive study of the copper content of many common plant and animal materials. Flinn and Inouye (68) were among the first investigators to attempt a discussion of the physiological aspects of copper in various organisms. They found a value of 26.0 milligrams of copper in one kilogram of cov liver. Cunningham (30) in 1931 published a comprehensive study of the copper content of many of the tissues and organs of different species, including a mature bovine, a newborn calf and a fetus. He found in general that the highest concentration of copper was present in the liver, kidney, heart, brain and hair, whereas the skin, lung, pancreas, spleen, and flesh contained small amounts of copper. He suggested that based on the wide distribution of copper in plant and animal tissue, it is possible that it may be a constituent of protoplasm. Hellwig and Quam (84) found in beef tissues that the highest concentration was in the liver, with decreasing amounts in the kidney, heart, cartilage, lung and spleen. The work of Schultze and Simmons (172) has shown that the highest relative accumulation of copper in the rat occurs in the kidney, liver, and bone marrow. The liver shows the greatest absolute retention of radioactive copper. On the unit weight basis, the kidney exhibited the highest accumulation of the organs analyzed (172). The daily copper requirement for various species has been given by Mitchell (134). A level of three parts per million in the ration is required by the rat.

Copper is not readily absorbed by the animal. Houk et al (94) have reported that rats on varying diets retained from 3.0 to 6.2 percent of the dietary copper. Lindow et al (116) found that 98 percent of the supplemental copper administered to the rat was excreted in the feces.

The rapid elimination of copper has been confirmed by Eden (52), who reported that 96 percent of the supplemental dose administered to the rabbit by stomach tube was eliminated in the feces and only one percent in the urine. Eden and Green (53) found that 17 percent of an injected dose of copper appeared in the urine within 48 hours and that 84 percent of the remainder was eliminated in the feces within four weeks.

The relation of increased copper level in the diet on the copper content of some animal products has been studied. Elvehjem et al (61) found that there was little difference in the copper content of milk from various sections of the country, and there was no change in the content of cow and goat milk when the copper level of the diet was increased fivefold the normal level. Remey (155) likewise found that there were no significant differences in the copper content of various types of milk. The copper content of the egg is not increased by the prolonged intake of copper supplements by the hen (59). The beneficial effects of copper supplementation on the wool of sheep has been reported in Australia (5).

Most of the more recent investigations concerning the mechanism of copper activity in the animal body have dealt with the distribution of copper in various tissues, the changes which occur in the blood under various pathological conditions, and the possible relations which may exist between copper and various vitamins and enzyme systems.

The identification of several enzymes as being complex copper-protein compounds (33, 103, 104, 108) has emphasized the importance of the catalytic activity of copper compounds. Schultze (169, 170) made the observation that copper is necessary for the formation and maintenance of cytochrome-C

oxidase activity in rat tissues. In copper deficiency the bone marrow shows a low cytochrome oxidase (170), and there is a diminished intensity of the spectral bands of cytochrome-A in rat tissues (25).

The occurrence in nature of copper-proteins has been reported by several investigators. Mann and Keilin (126, 127) have isolated two copper-protein compounds in mammals. Haemocuprein, a blue compound of the blood present in both the red corpuscles and serum, appears to account for all the copper in the corpuscles. The other protein substance, hepatocuprein, has been isolated from the liver and is colorless. The specific functions of these compounds are yet undetermined. The isolation of a copper-protein substance in milk has been reported by Dills and Nelson (42).

There have been reports in the literature that the copper content of the skin is closely related to the pigmentation of the skin (4). Sarata (164) has reported that the skin under pigmented hair has a higher copper content than that under colorless hair. Yoshikawa (195) has reported the essentiality of copper for the production of melanin.

It has been reported by Free (70) that the graying effect in rats could be due to either a lack of vitamins or could be caused by a deficiency of copper, as well as several other elements. Henderson and his coworkers (85) reported that supplementation of the diet with 100 micrograms of calcium pantothenate per day had no effect on preventing the graying of piebald rats on a copper deficient diet composed of whole milk, supplemented with iron and manganese, whereas additions of 50 micrograms of copper sulfate corrected the condition. Other workers have reported the relationship of pantothenic acid and achromotrichia. Unna and Sampson (186)

stated that doses of five, ten and twenty micrograms of calcium pantothenate were insufficient to prevent graying, whereas forty micrograms gave inconsistent results. Gyorgy and Poling (78) have reported that 75 to 100 micrograms of pantothenic acid daily caused definite restoration of pigmentation in five to seven weeks when administered to deficient rats. There have been other publications which have reported that copper deficiency is characterized by a graying of the hair of rats (31, 85, 101), rabbits (179) and ruminants (5, 31, 115). Neil and Nelson (101) observed that black rats fed a milk diet became gray and that supplements of copper salts would restore the original color. They confirmed the effect of copper on repigmentation of the hair of rats suffering from nutritional anemia. Copper resulted in complete restoration of color in about two months (101, 179). Smith and Ellis (179) reported that a deficiency of copper in the diets of rabbits resulted in anemia, graying of the hair, loss of hair and dermatosis. Cunningham (31) has observed that black cattle which have grazed for considerable time in copper deficient areas undergo a color change from black to muddy brown.

Several excellent reviews have been published on copper deficiency diseases (73, 135, 162, 181, 192). Russell (162) has emphasized the possibility that many copper deficiencies may in reality be molybdenum toxicity, as such a relation appears to exist.

In Australia, several disorders have been attributed to copper deficiency. Bennetts (11) has reported that "stringy" wool is the earliest indication of copper deficiency in sheep. When the degree of deficiency is insufficient to cause ataxia, there may be a retardation of growth and

development of lambs (12). Coast disease is alleviated by copper and cobalt (9). Enzootic ataxia, a disease of unweaned lambs, is characterized by a very low copper value of the liver and of a relatively low level in the blood and milk of affected ewes (15). The disease is prevented by the feeding of copper (13, 15). Falling disease in cattle is associated with a low copper status of pastures. An average of 2.1 parts per million of copper in the livers of cattle which have died of this disease has been reported by Bennetts et al (14). The value obtained for the normal animal was 122 parts per million. Copper supplements have given excellent results in health and production (10, 14, 16).

In 1944, Cunningham (31), in an extensive review of the copper problem, as it affects livestock in New Zealand, reported that the majority of reclaimed swamps, peaty soils, pumice soils, and pumice mixtures analyzed by him had proven to be copper deficient, ranging from 2.1 to 7.5 parts per million. The occurrence of "peat scours" in dairy cattle is characterized by a rough coat and a persistent, severe, debilitating scouring. The affected cattle are unthrifty and there is frequently much difficulty in getting cows with calf. In some areas there is a marked bone fragility in young calves, which, although not definitely proven to be the result of a lack of copper, is associated with a low copper intake. Beef cattle are not as severely affected as dairy cattle, and horses and pigs do well on the same pastures. In lambs, a severe copper deficiency is characterized by death or paralysis at birth or soon after. In other animals it is characterized by an incoordinated staggering gait. The disease is controlled by copper supplements.

The occurrence of "warfa" or "swayback" in lambs in North Derbyshire was reported in 1938 by Dunlop and Wells (50). The incidence of the disease is greatly reduced by copper treatment. The exact role of copper in this disease is not understood; however, the analysis of affected pastures have indicated that the disease is not a copper deficiency resulting from inadequate intake (17, 95, 96). Shearer et al (175) have indicated that although there is no direct relation between the blood copper value of the ewe and the incidence of "swayback" in her lamb, there is an incomplete transfer of copper to the fetus. Stewart et al (180) have stated that if the disease is due to a copper deficiency on pastures, which exceed five parts per million of copper and have a low molybdenum value, there must be some other factor than molybdenum which has affected the normal copper metabolism.

The occurrence of "licking disease" of cattle in areas of Holland was described by Sjollema (177) in 1933. The condition is apparently a copper deficiency in which the symptoms are anorexia, anemia, and a general loss of condition. In 1938 Sjollema (178) reported another copper deficiency affecting cattle and goats. The symptoms of the disease are diarrhea, loss of color in dark animals, and loss of weight. The copper content of the blood, liver and milk of affected animals was exceptionally low. Copper sulfate treatment gave favorable response. This has been confirmed by Nicolaisen and Seelbach (144).

Washburn (191) has reported a condition in cattle fitted for show on nurse cows which is characterized by simple or multiple symptoms, including lameness, crooked feet and legs, fragile bones, soft and eroded

bone articulation surfaces, and occasional sterility. Bulls are more susceptible than heifers or steers. When a level of 15 parts per million of copper was fed to the bulls on nurse cows the symptoms failed to develop. Watson and Smith (192) have reported the control of a disease called "salt pine", which is characterized by diarrhea and anemia, by the feeding of copper salts.

Neal et al (141) have reported an iron-copper disease in cattle, sheep, goats and swine in Florida which is characterized by loss of appetite, emaciation, weakness, pale tissues, constipation and diarrhea, a retardation of growth in young animals, and an impaired reproduction. The disease was known locally as "salt sick". Rusoff (161), in a comparison of the copper content of a newborn calf from a normal dam and a calf from a "salt sick" dam, found that with the exception of the skeletal tissues, the tissues of the normal calf contained less copper than the calf from the "salt sick" dam. Neal (140), in a later paper, found that the symptoms of copper deficiency included anemia, diarrhea, loss of appetite and depigmentation of the hair. Anemia does not occur in all species. The deficiency is attributed to forage containing less than three parts per million of copper, on a dry matter basis, although the deficiency may occur under some conditions with levels several times this amount. Davis et al (37) reported a naturally occurring copper deficiency in cattle grazed on some muck soils of Florida in which, in addition to the usual symptoms, there was an evidence of an abnormal bone metabolism resulting in rickets-like swellings of the long bones of calves and a rarification of the bones of older animals. This is also reported by Davis and Hannan (36).

The poisonous effect of copper has not been as extensively investigated as have various other phases of the behavior of copper in the animal organism. Eden (51), Maerland (139), and Boughton and Hardy (18) have clearly described the symptoms of the toxicity. The effects of administering copper salts to animals over periods of time are reported by many investigators (67, 80, 86, 146, 147, 151, 165).

The beneficial effect of feeding supplemental copper to young foals and pigs has been reported. In an experiment with growing foals, Cupps and Howell (32) observed that in control animals, receiving eight parts per million of copper in the ration, there were lesions on the articular cartilage of the alanto-occipital, elbow, knee, and back joints. Animals receiving 100 parts per million copper had an eroded area only at the elbow joint. The role of copper in preventing or decreasing these lesions is not clear. Urbanyi (187) in an extensive investigation with a large number of sows and their young found that the feeding of a supplement of iron and copper to the gravid sows during the last third of pregnancy prevented or cured the anemia of the sows and produced young which had average weights of four to five percent higher than usual. There was also a greater resistance to disease in these young.

The biological importance of molybdenum in animal and plant nutrition has become more generally recognized in recent years. Ter Meulen (131) in 1932 pointed out the wide occurrence of the element in fertile soils and plant materials. The distribution of the element in nature has been reported by many investigators (40, 41, 43, 44, 47, 123, 157). Molybdenum, although not proven to be essential for animals, was reported

by Arnon and Stout (6) to be required for the growth of higher plants. Hoagland (92) has presented a review of the evidence of the essentiality of the element for plant growth. Ferguson et al (65) have observed that molybdenum is taken up by vegetation under alkaline soil conditions and that very little is taken up under acid soil conditions. Beath and co-workers (8) found that vegetation from cretacious shales of Wyoming absorbed molybdenum in varying amounts, reporting one sample which contained 317 parts per million. Barley containing 89 parts per million of molybdenum was grown by these workers on soils fertilized with sodium molybdate. Analysis of some forage from Florida muck areas have indicated levels as high as 80 parts per million of molybdenum (35). Alfalfa pastures containing 36 parts per million have been reported in Kearn County, California (19). Robinson and Edington (157) have reported the analysis of widely varied vegetation from different parts of the country. Vegetation containing as much as 137 parts per million of molybdenum trioxide has been reported in the high selenium areas of Columbia (157).

The presence of molybdenum in soils has been reported by many authors (63, 91, 97, 113, 156). The molybdenum content of phosphate rock in Florida has been reported in the literature (91, 97, 156). Robinson (156) found values ranging from 5 to 31 parts per million of molybdenum trioxide in various samples of Florida phosphate rock.

The literature previous to 1944, with regard to molybdenum, has been extensively reviewed by Fairhall et al (62). The toxicity of various compounds of the element to the guinea pig and rat were investigated by these authors. Animals fed molybdenite remained well and gained weight, whereas

those fed molybdenum trioxide developed anorexia, became quiet and listless, lost weight, and in certain groups the fur of the animals became harsh and rough. These investigators found that the greatest storage of molybdenum is in the kidney and bones. The excretion and absorption of molybdenum are rapid, indicating the transitory nature of the storage. Doses of 1200 to 6000 milligrams of molybdenum per kilogram of body weight when fed to the animals invariably proved fatal. Maresh and co-workers (128) reported the lethal dose of sodium molybdate for rats to be between 114 and 117 milligrams of molybdenum per kilogram of body weight. Doses up to 300 milligrams of molybdic acid were not toxic to the rabbit when fed by mouth (137).

The effects of high levels of molybdenum in vegetation on the health of grazing animals has been receiving increasing attention. According to Robinson and Edgington (157), the toxic level of 20 parts per million in vegetation set by the British (112) is too high. These workers indicate that borderline cases may occur with much lower levels. In evaluating the work of various English workers, Russell (162) suggested that the copper deficiency syndrome in cattle may be caused by an excess of molybdenum in the forage.

The earlier reports on the relationship of copper and molybdenum were concerned with the scouring produced on the so-called "teart" pastures of England. Ferguson et al (65) and Muir (136) have described teart pastures as those pastures containing more than 14 parts per million of molybdenum, and non-teart pastures as those containing less than six parts per million, whereas pastures containing from 6 to 14 parts per million are

classified as potentially teart. Ferguson et al (65), Lewis (112), Ferguson (63) and Lewis et al (113) have reported that the scouring of cattle in their area could be traced to the molybdenum content of the "teart" pastures. Some investigators (63, 65) have produced the same symptoms by the administration of molybdenum salts. These investigators also found that copper sulfate given as a drench had a therapeutic effect, preventing and curing these symptoms. Animals on affected areas show abnormally high storage of the element in the liver. Bush sickness, a disorder of cattle in New Zealand, is similar to molybdenum toxicity (7).

In 1947, Britton and Goss (19) confirmed the findings of the English workers and observed several uncomplicated cases of toxicity. Affected cattle became emaciated and exhibited an intense liquid diarrhea. There were changes in the color of the coat and a marked anemia. There was a pronounced jugular pulse on exertion and a weakness or stiffness being usually apparent. Prolonged purgation sometimes resulted in death to the animal. The observations indicate that young cattle are more susceptible than adult animals and that dairy animals are more susceptible than beef animals. It is reported that sheep are rarely affected and horses and swine are resistant to the toxicity. Beath and co-workers (8) found that barley containing 89 parts per million of molybdenum fed to livestock caused an erosion of the long bones and other pathological symptoms which are similar to those produced by cereals high in selenium. It was also demonstrated by these workers that the drenching of yearling calves daily with 100 milligrams of molybdenum, as sodium molybdate, until 18.3 grams were administered resulted in a loss of weight and pathological

changes. The elimination of molybdenum injected intravenously is primarily through the urine (2, 21, 22). There is an accumulation in the liver of intravenously injected ammonium molybdate and sodium molybdate (22). Ferguson et al (64) found that herbage high in molybdenum fed to milking cattle resulted in injurious action, causing a failure in milk production, loss of condition and even death. The herbage did not affect horses, although sheep were affected. Cattle developed the same pathological symptoms when fed molybdenum. Dick and Bull (39) have reported a low level of copper in the liver of cattle and sheep receiving molybdenum treatment over a prolonged time. The amount of copper present in the liver was reduced even though copper was added to the ration.

Teresi et al (182) reported that molybdenum is not essential in the growth of the rat unless the requirement is less than 0.5 micrograms per day. Neilands et al (142) fed high levels of sodium molybdate to 21-day-old rats which were placed on a purified ration, without gross pathological changes or effect on the blood copper. In a tracer study with radioactive molybdenum (Mo^{99}), these authors found that in periods up to two days most of the dosage was distributed in the stomach, intestines, feces and urine. At the end of two days the kidney and bone contained higher levels per gram of tissue. Copper given simultaneously did not effect the distribution. Investigation of the formation of insoluble copper molybdate as a probable cause of the poor absorption of sodium molybdate was conducted by these authors (142).

The diarrhea in cattle grazing in "teart" areas has been shown to be due to an excess of soluble molybdates (46, 65, 66, 112). The catechols

present normally in the digestive tract of ruminants prevents the excess growth of microorganisms; however, it has been shown, in vitro, that molybdates reduce the bacteriostatic activity of the catechols by forming complexes (119). The theory is advanced that with the removal of control, bacterial activity becomes excessive and results in diarrhea. The probability is that copper cures the condition by its simple control of bacterial activity (118). Neilands et al (142) found that catechol given simultaneously increased the absorption of Mo^{99} in the tissues and indicated that it may delay the elimination of molybdenum.

Schmidt and Greenberg (166) have published an extensive review concerning the occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism. The percentage of phosphorus, as reported by various authors, in the heart, kidney, liver, lungs, muscle, spleen, skin and skeletal tissue is tabulated. In 1944, Green and Colowick (75) presented a review of some of the more recent publications which have primarily dealt with the fate of the phosphorus radical in metabolism. The physiology of the bone is systematically reviewed by McLean (122).

Radioactive phosphorus has had so far the most extensive application of the radioactive isotopes (88). As a result of the importance of phosphorus in the animal organisms, the isotope has had both extensive application in both the study of inorganic and organic metabolism. The work of Chiewitz and Hevesy (23) in 1935 was one of the earliest experiments in which radioactive phosphorus (P^{32}) was applied as an indicator.

Approximately 80 percent of the phosphorus of the body is present in the bone and teeth (129). Gilbert (73) has indicated that 70 to 80

percent of the phosphorus of the body is present in the skeleton, 10 percent in the muscle, 10 percent in the nervous system, and that the remainder is widely distributed as a component of every cell. The investigations of Hevesy (87, 88) have developed data which indicate that, in comparison to a large portion of the phosphorus of the liver and kidney, only a minute portion of the skeleton or brain phosphorus is renewed or replaced within a few hours. Hahn et al (79) have indicated that the average time a phosphorus atom spends in the animal system is approximately thirty days. In experiments of short duration, those limited to several hours, it was found that the skeleton and muscle of rats contained almost the same amount of radioactive phosphorus (88). In experiments of extremely long duration, labeled phosphorus is stored primarily in the bone (88). Hevesy (87) has indicated in another paper that 92 percent of the P^{32} present in the body was stored in the skeleton. In a series of experiments with rabbits and frogs, Hevesy et al (89) were able to conclude, by maintaining the inorganic phosphorus in the radioactive plasma at a constant level through repeated administration of labeled phosphorus, that 29 percent of the epiphysis of the femur and tibia was replaced within fifty days, while only seven percent of the diaphysis. The phosphatides of the bone and marrow were, according to these investigators, entirely renewed during this period. The exchange of phosphorus from the blood plasma to bone phosphate is extremely rapid. By the use of radioactive phosphorus, injected and ingested, the extent and rate of replacement of bone tissue has been determined (87, 89, 90).

Warren and Cowing (190) found in distribution studies with mice,

rats and rabbits that the percentage of partition between the various organs of the body varies with the different species, but that there is a material degree of absorption in the spleen, liver, kidney, and bone in all three species. The work of Born (17) has furnished more extensive information relative to the distribution of phosphorus in various bones. In these investigations the experimental rats were dissected 72 hours after they had been given an injection of radioactive phosphorus in the form of sodium phosphate. The largest amount of total radioactive phosphorus was found in the skull, although there was appreciable uptake by various other bones. Using very young rats, Conceiro (28) found that in periods up to thirty days after injections of di-sodium phosphate, containing radioactive phosphorus, most of the absorbed phosphorus was deposited in the bone and relatively small quantities were found in the muscle, liver, kidneys and brain.

The absorption of radioactive phosphorus by the bone is extremely rapid after injection of the substance into the circulatory system. According to many investigators (23, 27, 45, 87, 124) the greatest part of the bone activity is due primarily to the uptake of P^{32} by the so-called apatite structure of the bone. The findings of Grodzenkii and Il'ina (76) have shown that radioactive phosphorus is deposited in the bone from four to sixty-five hours after administration. This is in agreement with the work of other investigators. The work of Cohn and Greenberg (26) has shown that the major deposition of injected or ingested radioactive phosphorus occurs with the first eight hours after administration, being the most rapid the first two hours. According to their studies, phosphorus is

retained by the tissues in the following decreasing order: bone, muscle, liver, stomach and small intestines, blood, kidney, heart, lungs and brain. Based on a unit of fresh weight, phosphorus is retained by the tissues according to the following decreasing order: bone, liver, stomach and small intestines, heart, kidney, lungs, muscle, skin and brain. These experiments demonstrate that the first 24 hours in all probability are the most important for studying the movement of a single dose of administered radioactive phosphorus.

The study of the mode of entrance of phosphorus into the bone has led to the concept that there are two functions within the bone; one fraction is basically a labile functional organic structure, which is continually exchanging with the phosphorus of the plasma, and the other fraction is a stable inorganic structural formation which receives its phosphorus in increments from the labile fraction (125, 185, 190). According to Manly et al (125), a rational explanation of the behavior of calcified tissue following the administration of a dose of radioactive phosphorus must be based on such a concept.

In an investigation with rats, Cohn and Greenberg (26) found that 40 percent of the ingested radioactive phosphorus remained unabsorbed eight hours after administration. During the first eight hours, about 20 to 30 percent of the absorbed P^{32} is excreted in the urine, whereas three percent of the administered dose is eliminated in the feces. Hahn et al (79) found that within 27 days, 45 percent of the dose of radioactive phosphorus administered to rabbits was excreted in the urine and 11.5 percent in the feces.

The hemoglobin content of the adult rat averages 14.44 grams per 100 cubic centimeters, whereas the newborn rat has a reported value of 11.86 grams per 100 cubic centimeters (72). Croskoff et al (29) and Wintrobe and co-workers (194) have reported a slightly higher hemoglobin value for the female than for the male, while Reich and Dunning (153) found a lower value for the female. The accepted normal average value for the bovine is 12.03. The average hemoglobin content of various species is given by Dukes (49).

PROGRAM OF EXPERIMENT

The rat has been used as the experimental animal to determine the effect of various levels of copper and molybdenum on the growth, physical appearance, reproduction, hemoglobin values, and accumulation of various minerals in the liver and bone. General observations have been made throughout the experiments. Chemical analyses have been used to determine the level of copper and molybdenum in the liver and the molybdenum in the bone. Hemoglobin values have been determined on representative animals of each group. The effect of copper in hair pigmentation and its relation to pantothenic acid has also been investigated. The effect of a high level of molybdenum has been demonstrated on animals raised on a low copper-low molybdenum ration. Selected animals have been autopsied to determine the physiological effect of a high molybdenum-low copper ration.

Tracer studies with radioactive isotopes have been used to determine the distribution and excretion of copper, molybdenum and phosphorus under normal and restricted conditions of diet and the influence of the elements upon each other. The bovine has been used as the experimental animal in radioactive copper, molybdenum, and phosphorus experiments to study the distribution in the tissues, the excretion, and the retention and absorption in the blood. A study of the accumulation of molybdenum in the liver has also been conducted with this species. A biopsy technique has been employed. The rat has been used to study the rate of accumulation and distribution of copper and molybdenum in select tissues, the rate of excretion of the elements, and the percent of radioactive phosphorus remaining in the gastro-intestinal tract at the end of 24 hours. It has been used in comparative

studies to determine the rate of absorption of phosphorus by selected tissues in the young and mature rat and the effect of fasting and non-fasting before the administration of radioactive phosphorus. The rabbit has been used to determine the tissue distribution of administered copper.

GENERAL EXPERIMENTAL PROCEDURE

Radioactive isotopes were employed for tracer studies. The isotopes were supplied by the Clinton Laboratories and Oak Ridge National Laboratory and were obtained on allocation from the United States Atomic Energy Commission.

Copper 64, an isotope of inert copper, which has a half-life of 12.8 hours and emits beta particles, positrons and annihilation gamma rays (173), was used in the copper studies. The irradiation unit, consisting of 0.32 gram of pure copper wire, was transferred from its shipping container to a tall form 200 milliliter beaker and dissolved in six milliliters of eight normal nitric acid. A magnetic stirrer was used to provide agitation while slowly adding two normal sodium hydroxide. The acidity of the solution was adjusted so that further neutralization would result in precipitation of the hydroxide. The solution had a pH of approximately three.

The isotope of molybdenum, Mo^{99}, has a half-life of 67 hours and emits beta and gamma radiation (173). The isotope was obtained as an irradiation unit consisting of 10 grams of molybdenum trioxide (MoO_3), with an initial activity of about 40 millicuries. The material was removed from its shipping container and carefully transferred to a 400 milliliter beaker and 35 milliliters of 14.3 percent sodium hydroxide were added. Agitation was provided by a mechanical stirrer in order to obtain a clear solution in a relatively short time. The clear solution was diluted with distilled water to the required volume. The pH of this solution was approximately seven.

The radioactive phosphorus isotope, P^{32}, has a half-life of 14.3 days and is a beta emitter (173). The irradiation unit consists of 0.25 gram of P^{31} in the form of di-sodium phosphate or ortho phosphoric acid. This material was diluted with distilled water to the required volume.

The determinations of the half-life of the isotopes have been in good agreement with the reported values. Adequate health precautions were taken to insure minimum danger. Radiation monitoring equipment was utilized to minimize contamination and exposure.

A rapid method of pseudo-wet ashing has been developed to facilitate the handling of large numbers of samples. A representative sample of the tissue, up to 50 grams, was placed in a 400 milliliter beaker and concentrated nitric acid was added. The amount of acid was related to the weight and nature of the sample. A 50-gram sample required 40 milliliters, whereas a sample of several grams required 10 milliliters or less. After soaking for about 10 minutes, the beaker was transferred to a hot plate and gentle heat was applied. After solution of the sample, which in some instances required further addition of acid, the volume was evaporated to approximately 15 milliliters and the beaker transferred to a steam bath for evaporation to dryness. The residue was washed into a separatory funnel with hot water, after which the beaker was rinsed with two 10-milliliter portions of iso-amyl alcohol, which in turn were added to the separatory funnel. The separatory funnel was shaken gently and the aqueous layer removed to a volumetric flask. The alcohol layer was washed with five milliliters of warm water, which were also added to the volumetric flask. Experience has shown that the alcohol layer does not contain any of these

radioactive tracers.

Radioactive measurements were made on the solution obtained by the pseudo-wet ashing process. The measurements were made with a Geiger-Mueller apparatus, equipped with a dipping type counter or the mica window bell jar type counter. Two instruments were used, one manufactured by the Instrument Development Laboratories, and the other by the Cyclotron Specialties Company. Calibration curves were determined daily and decay corrections were applied to the values obtained.

Liver biopsy samples were taken from the bovine by the following method: After the subcutaneous injection of two milliliters of a two percent procaine solution in the proposed area of the penetration, a small incision was made in the hide and muscle between the twelfth and thirteenth ribs. A trocar, having a diameter of .3 centimeter and a length of 16 centimeters, was passed through the incision into the peritoneal cavity. The stilet was then removed and the cannula was turned as it was pushed in a forwardly and downward direction toward the liver. After a reasonable penetration of the liver tissue, the end of the cannula was sealed and the instrument withdrawn from the animal. The plug of liver in the cannula was transferred to a small tared beaker, weighed, and then ashed by the pseudo-wet ashing method.

Half-gram liver samples were prepared for quantitative analysis by the method of Linder and Harley (114), which was modified by repeated additions of hydrogen peroxide until the solution remained colorless after continued heating. Copper was determined by the carbamate method of Eden and Green (54), modified by extraction of the copper diethyl-dithio-carbamate

from the aqueous solution with 25 milliliters of iso-amyl alcohol. Molybdenum was determined by the thiocyanate method (163), using ethyl ether as the solvent for the extraction. The Cenco spectrophotometer, modified to use a 15 centimeter length cuvette, was used in both of these determinations.

Rat bones were prepared for quantitative analysis by cleaning extraneous tissue from them, drying at 100 degrees for twelve hours, and dissolving in 10 milliliters of a concentrated acid solution (five milliliters of hydrochloric acid to one milliliter of nitric acid). The solution was evaporated to remove the excess acid and the residue was then taken up with 50 milliliters of distilled water. This solution was filtered and made to volume. Molybdenum was determined by the thiocyanate method.

Rat blood was obtained for hemoglobin determinations by anesthetizing the animals with ethyl ether and cutting off the tip of the tail. Hemoglobin values were determined by the acid-hematin method of Cohen and Smith (24).

The excretion studies with the bovine were conducted by keeping the animals in special digestion stanchions, constructed so that the urine and feces were collected separately with little danger of being mixed. The animals were brushed and rubbed daily to maintain muscular tone. The excretion studies with the rat were conducted in special metabolism cages in which the entire excretion was collected on Whatman No. 2 filter paper. The fecal matter was separated for analysis by mechanical removal from the filter paper containing the urine.

Various solutions were orally administered to the rats by means of a small hypodermic syringe fitted with a blunt needle.

All animals used in this study were obtained from the Nutrition Laboratory of the Florida Agricultural Experiment Station at Gainesville, Florida.

In certain instances it has been necessary to calculate the weight of the blood, liver and bone of an animal. The weight of blood in the rat has been calculated as being 7.0 percent of the body weight (72), and that of the bovine as 7.7 percent (49). The weight of the bone in the bovine is calculated as 10.5 percent of the body weight (197). The weights of the livers of animals which have not been sacrificed are based on the weights of the liver of comparable animals which have been sacrificed at the Nutrition Laboratory of the Florida Agricultural Experiment Station at Gainesville, Florida.

EXPERIMENT 1

Experimental Procedure and Results

To determine the effect of various levels of copper and molybdenum in a simplified ration on the general metabolism of the rat, 127 21-day-old white, black and piebald rats were selected from the stock colony. These rats were divided into four groups which have been designated as I, II, III, and IV. For convenience the rations of these groups are designated by the same number. The composition of these rations are given in Table 1.

TABLE 1. Composition of Rations
(in grams)

	I	II	III	IV
Whole Milk Powder (Klim)	3415.0	3415.0	3415.0	3415.0
Sucrose	3379.0	3380.0	3380.0	3381.0
Sodium Chloride	34.0	34.0	34.0	34.0
Ferrous Sulfate	0.136	0.136	0.136	0.136
Manganous Sulfate	0.06	0.06	0.06	0.06
Thiamine Chloride	0.023	0.023	0.023	0.023
Sodium Molybdate	1.18	--	1.18	--
Copper Sulfate	0.506	0.506	--	--

The copper and molybdenum content of the ration, as determined by chemical analysis, is given in Table 2. Pyrex-distilled water was supplied to all

TABLE 2. Copper and Molybdenum Content of Rations
(in parts per million)

	I	II	III	IV
Copper	32	24	1	1
Molybdenum	80	below 1.0	80	below 1.0

animals throughout the experiment.

The animals of this experiment were maintained on a specified ration for 57 days and were weighed at definite intervals. The summary of the weights of the females and males of each group are given in Table 3. Figures 1 and 2 are graphic representations of the growth curves which are presented for clarity. These figures and the table do not indicate the number of fatalities, but are average weights of the living rats in the groups. Fourteen animals died in Group III and one in Group IV, but none in Groups I and II. In Group III, 43.5 percent of the female rats died, in comparison to 17.4 percent of the males. Six additional rats of Group III were sacrificed for observations.

The animals in Groups I and II were of normal appearance throughout the experiment. The animals in Groups III and IV were normal in appearance during the first two and one-half to three weeks. After this there was a lightening of the haircoat (Figures 3 and 4) in the black and piebald rats. The graying followed a distinct pattern. The animals retained a dark stripe in the center of the back extending from the nose to the tail for a considerable time after the graying of the remainder of the haircoat. Figure 3 represents the pattern of graying at the end of three to four weeks of experiment, and Figure 4 is a picture of the typical black rat after six weeks. Toward the end of the experiment the animals of Groups III and IV were stunted in appearance. The affected animals of Group III became emaciated, anemic and weak, and showed a retarded skeletal development with poor calcification. They developed diarrhea and lost considerable weight during short periods of time. In severe cases, the

TABLE 3. Growth Studies of Rats Maintained on Simplified Rations With Varying Levels of Copper and Molybdenum (Average Weight and Deviation in grams)

Group	No. of Rats	Sex	Age in Days					
			21	25	29	32	36	39
I	16	Female	37.4 ± 4.1	41.7 ± 3.9	48.9 ± 5.2	54.7 ± 7.4	61.7 ± 6.6	62.6 ± 6.9
II	9	Female	36.0 ± 3.8	42.1 ± 4.2	50.0 ± 5.1	58.4 ± 4.3	66.8 ± 6.0	70.3 ± 7.2
III	23	Female	36.9 ± 3.0	40.5 ± 3.5	47.2 ± 4.4	50.6 ± 4.9	56.4 ± 5.5	59.2 ± 6.5
IV	21	Female	38.3 ± 4.4	43.0 ± 5.2	49.8 ± 5.1	56.7 ± 5.6	64.0 ± 7.2	67.0 ± 7.1
I	14	Male	39.4 ± 2.8	44.9 ± 4.3	51.3 ± 5.2	56.1 ± 6.3	63.4 ± 5.8	68.5 ± 7.2
II	8	Male	36.5 ± 2.8	41.8 ± 3.8	48.5 ± 4.4	55.0 ± 5.5	64.3 ± 6.9	64.7 ± 6.9
III	23	Male	39.2 ± 4.0	43.6 ± 4.8	52.3 ± 5.5	56.8 ± 7.3	64.0 ± 9.3	68.1 ± 9.9
IV	13	Male	37.6 ± 3.0	43.3 ± 3.7	51.3 ± 5.4	56.6 ± 6.8	63.0 ± 6.7	66.9 ± 7.0

Group	No. of Rats	Sex	Age in Days					
			43	50	57	64	71	78
I	16	Female	70.4 ± 6.1	79.3 ± 8.1	94.6 ± 12.2	101.2 ± 12.3	113.8 ± 11.9	125.5 ± 11.5
II	9	Female	81.3 ± 7.4	97.9 ± 6.5	115.0 ± 10.8	124.4 ± 7.6	134.7 ± 8.5	144.4 ± 9.5
III	23	Female	63.3 ± 9.2	67.7 ± 8.7	72.3 ± 8.3	74.7 ± 10.5	73.4 ± 11.9	72.9 ± 17.3
IV	21	Female	73.9 ± 8.7	78.6 ± 12.9	85.2 ± 13.3	90.0 ± 17.8	98.3 ± 20.1	109.0 ± 19.6
I	14	Male	75.3 ± 8.6	88.3 ± 10.9	103.3 ± 16.1	116.1 ± 17.8	130.2 ± 17.6	147.2 ± 19.2
II	8	Male	73.7 ± 7.4	91.7 ± 7.5	110.5 ± 8.7	131.2 ± 11.0	144.4 ± 11.3	160.2 ± 16.4
III	23	Male	74.3 ± 10.6	82.0 ± 13.9	87.6 ± 19.0	99.2 ± 19.4	112.0 ± 19.9	121.1 ± 20.2
IV	13	Male	72.7 ± 7.3	80.4 ± 10.7	87.5 ± 7.3	92.8 ± 7.6	99.1 ± 7.3	110.0 ± 8.8

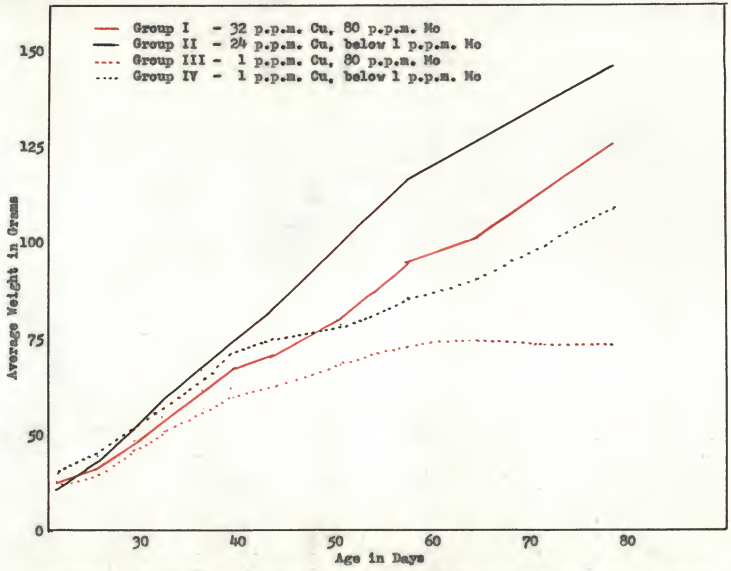

FIGURE 1. Graphic Representation of Average Weights of Female Rats Maintained on Simplified Rations With Varying Levels of Copper and Molybdenum

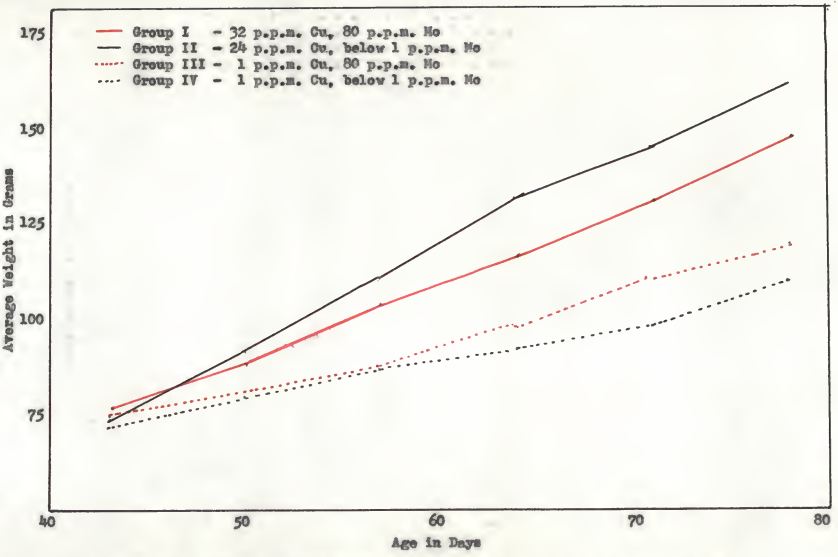

FIGURE 2. Graphic Representation Average Weights of Male Rats Maintained on Simplified Rations With Varying Levels of Copper and Molybdenum

haircoat became rough and alopecia was apparent. In some instances there was severe lacrimation of the eyes and in others there appeared to be some degeneration of the liver. The contrast between an average animal of Group II and a severely affected animal of Group III is shown in Figure 5. These animals are of the same age.

The hemoglobin values of representative animals of each group, without regard to sex, were determined when the animals were approximately 60 days of age. These values are given in Table 4.

The effect of molybdenum on the young copper deficient rat was studied by transferring five 90-day-old animals which had been raised on Ration IV (low copper-low molybdenum) to Ration III (low copper-high molybdenum). Five animals of the same age and treatment were continued on Ration IV as controls. Within 27 days, all of the animals on the high molybdenum ration were dead. The weights of these animals during the trial are given in Table 5.

Representative animals of each of the groups were sacrificed and the livers and the femur, tibia and fibula bones were taken for analysis. The molybdenum and copper values of the livers and the molybdenum values of the leg bones are given in Table 6.

Seven 90-day-old female rats from Group IV were bred to males from the same group. The males were removed from the breeding cages after 15 days. One litter was born, perfectly formed, but dead. Six females of the same age from Group II were bred to males of Group II and gave birth to six normal litters.

FIGURE 3. Typical Copper Deficient Rat After Three Weeks

FIGURE 4. Typical Copper Deficient Rat After Five to Six Weeks

FIGURE 5. Normal Rat (Left) and Rat With Severe Molybdenum Toxicity (Right)

TABLE 4. Hemoglobin Values of 60-day-old Rats Maintained on Simplified Rations With Varying Levels of Copper and Molybdenum (grams per 100 milliliters of blood)

Animal No.	Group I	Group II	Group III	Group IV
1	11.0	11.8	8.8	8.3
2	11.2	11.7	8.2	8.0
3	11.2	14.4	9.1	10.3
4	11.5	13.2	6.4	5.6
5	11.5	13.0	6.2	7.8
6	12.4	14.3	7.0	7.6
7	12.0	12.3	5.3	6.2
8	11.2	12.6	6.8	6.6
9	13.0	13.1	7.0	8.5
10	--	14.2	7.6	--
11	--	--	8.0	--
12	--	--	6.3	--
Average Weight and Deviation	11.7 ± 0.5	13.1 ± 0.8	7.2 ± 0.9	7.7 ± 1.0

TABLE 5. Effect of Molybdenum on Rats Raised on a Low Copper Simplified Ration

Treatment	Animal No.	Weight in Grams				
		Initial	7 days	14 days	21 days	28 days
Ration IV	1	127.6	151.0	156.8	158.4	154.4
	2	113.2	127.8	138.8	144.0	148.4
	3	118.8	146.2	162.5	169.4	180.0
	4	119.4	131.5	142.4	151.2	156.8
	5	121.4	125.8	142.1	149.0	155.0
Ration III	6	143.6	88.6	81.4	77.4	60.0*
	7	110.4	96.0	84.0	75.8	65.0*
	8	123.6	62.8	55.8*	--	--
	9	96.6	85.2	63.8	62.0*	
	10	93.2	75.8	66.8	59.6*	

* Dead

TABLE 6. Mineral Accumulation in the Liver and Bone of Animals Maintained on Various Levels of Copper and Molybdenum in the Simplified Ration

Group	Animal No.	Copper in the Liver	Molybdenum in the Liver	Molybdenum in the Bone
I - High Copper High Molybdenum	1	33.9	5.3	13.9
	2	47.3	12.3	9.1
	3	28.4	10.9	—
	4	76.3	14.2	—
	5	33.9	12.9	13.8
	6	25.3	—	—
II - Normal Copper Low Molybdenum	1	43.9	1.0	0.1
	2	51.7	1.5	0.1
	3	57.8	1.0	0.2
	4	73.5	2.0	0.2
	5	34.2	1.0	0.3
	6	32.3	1.0	0.1
	7	25.2	2.0	0.2
III - Low Copper High Molybdenum	1*	1.0	6.4	—
	2	14.0	4.0	6.6
	3	22.6	13.2	1.3
	4*	1.0	35.7	12.4
	5	4.5	4.4	10.0
	6	10.4	6.3	9.5
	7	6.0	13.6	12.0
IV - Low Copper Low Molybdenum	1	8.5	2.6	0.1
	2	12.5	1.6	—
	3	3.9	1.1	0
	4	11.8	0.9	1.4
	5	7.3	1.5	0
	6	9.6	1.0	0
	7	11.5	2.1	0.2
	8	3.2	4.6	0
	9	2.6	1.2	0.3

* Animal which died on experimental trial

Discussion and Conclusions

High levels of molybdenum are toxic to the rat when adequate copper is not present. The growth study of rats maintained on a simplified ration has indicated that 80 parts per million of molybdenum in a ration deficient in copper will result in a severe diarrhea, weakness, alopecia, and a rough haircoat. It may also result in degeneration of the liver, a retarded skeletal development with poor calcification of the bones, a severe lacrimation of the eyes, and death to the animal.

The anemia and graying of the haircoat, observed in animals of Group III (low copper-high molybdenum ration), was the result of a copper deficiency which may in part have been the result of the high molybdenum level of the ration. This was indicated by the reduction of copper in the livers of the animals of this group and was further indicated by the negligable amount found in the livers of two animals which died as a result of the toxicity. The graying, although occurring in both Group III (low copper-high molybdenum ration) and Group IV (low copper-low molybdenum ration) did not occur any earlier in Group III. A degree of depigmentation was apparent in both groups at the end of approximately three weeks. The average hemoglobin value of the blood of the animals of Group III was reduced below the normal value and was slightly lower than that of animals of the same age which were maintained on a low copper ration containing 1.0 part per million of molybdenum.

The high mortality occurring among the animals of Group III emphasizes the toxicity of the ration. The female animals appear to be more susceptible than the male. The toxicity of molybdenum is further emphasized

by the 100 percent mortality which occurred within 27 days after the animals which were raised from weaning age (21 days) to 90 days of age on a low copper-low molybdenum ration (Ration IV) were changed to a low copper-high molybdenum ration (Ration III). In all cases there was a sudden loss of weight accompanied by severe diarrhea. There was a high level of molybdenum present in the livers and bones of the animals in Group III.

The retardation of growth and the general effects of 80 parts per million of molybdenum were to a large extent overcome by 32 parts per million of copper in the ration (Group I). The growth of the animals of Group I was almost equal to that of the control animals of Group II, which received a level of 24 parts per million of copper and less than one part per million of molybdenum in their ration. The hemoglobin values of the animals of Group I were lower than those of the control group (Group II) but were much higher than those obtained for Group III (low copper-high molybdenum) and Group IV (low copper-high molybdenum). The copper level in the liver is comparable to that of the normal group. There is a storage of molybdenum in the liver and bone.

The animals of Group IV were retarded in growth and showed no signs of abnormalities in bone structures or development of the organs. There is evidence that copper deficiency results in a failure to reproduce. This is in all probability due to either a delay in sexual maturity or an impairment of the reproductive organs. The animals in this group have a lower average growth than those of any other group. Copper deficiency in the rat is characterized by anemia. Some of the older animals, not mentioned in Table 4, have had hemoglobin values as low as 2.9 grams per 100 milliliters

of blood. The average hemoglobin value at 60 days of age is approximately 50 percent of normal. The copper content of the liver is appreciably reduced as can be seen in Table 6. There is evidence of some storage of molybdenum in the liver.

A graying of the black and piebald rat occurs on a copper deficient diet and is well illustrated in Figures 3 and 4. There is a characteristic pattern in which there is a considerable delay in depigmentation of a narrow stripe which extends across the center of the back from the nose to the tail. This stripe may remain dark for two or three weeks after the remainder of the haircoat has turned silvery gray or brown. The role of copper in pigmentation is not understood.

EXPERIMENT 2

Experimental Procedure and Results

The effect of molybdenum added to the commercial ration (Staf-O-Life Dog Food, manufactured by the Royal Staf-O-Life Mills, Memphis, Tennessee) was studied by placing 142 21-day-old white, black and piebald rats from the stock colony on various levels of molybdenum, in the form of sodium molybdate. The dog food was ground in order to assure a more even distribution of the molybdenum in the ration. To eliminate the incorporation of additional copper from metallic sources, all rations were prepared by grinding in a mortar and pestle. The copper content of the ration within each group varied from 13 to 60 parts per million. Differences were noted in each bag of feed. The animals of Group V received an average of 31.5 parts per million of copper, Group VI 35.6, Group VII 32.7, and Group VIII 43.6.

The animals were divided into four groups which have been designated as Groups V, VI, VII, and VIII. For convenience the rations of these groups are designated by the same number. Group V was fed the normal ration without any supplement, Group VI received 80 parts per million of molybdenum, and Groups VII and VIII received 120 and 160 parts per million of molybdenum, respectively. These animals were maintained on the specified ration for 57 days and were weighed at definite intervals. The number of rats in each group and the average weights throughout the experiment are given in Table 7. All animals of this experiment were normal in appearance.

The hemoglobin values were determined on representative rats of each group at 80 days of age and are reported in Table 8. Representative animals

TABLE 7. Growth Studies of Rats Maintained on Commercial Rations Supplemented with Varying Levels of Molybdenum (Average Weight and Deviation in grams)

Group	No. of Rats	Sex	Age in Days					
			21	25	29	32	36	39
V	21	Female	40.7 ± 3.8	53.1 ± 2.6	67.7 ± 4.3	81.3 ± 4.7	96.7 ± 6.5	108.8 ± 6.2
VI	26	Female	35.4 ± 4.9	48.3 ± 5.6	63.3 ± 5.6	75.0 ± 6.4	91.1 ± 7.7	100.0 ± 7.8
VII	13	Female	34.1 ± 2.1	44.0 ± 2.6	59.2 ± 3.7	72.9 ± 6.6	84.8 ± 4.6	92.1 ± 4.5
VIII	10	Female	40.2 ± 3.0	54.0 ± 3.1	66.9 ± 3.0	80.9 ± 3.7	95.0 ± 4.5	105.6 ± 5.4
V	19	Male	42.3 ± 3.6	56.9 ± 3.8	76.2 ± 4.8	92.7 ± 6.5	114.2 ± 7.1	130.5 ± 7.4
VI	25	Male	40.8 ± 4.3	56.9 ± 6.8	75.5 ± 8.6	96.8 ± 12.7	107.8 ± 12.6	120.5 ± 12.2
VII	13	Male	34.5 ± 3.7	49.5 ± 5.6	65.2 ± 7.7	79.2 ± 10.5	98.7 ± 12.4	110.9 ± 16.0
VIII	15	Male	43.3 ± 2.5	59.5 ± 3.0	76.8 ± 4.6	93.7 ± 8.0	111.4 ± 7.7	125.8 ± 11.4

Group	No. of Rats	Sex	Age in Days					
			43	50	57	64	71	78
V	21	Female	119.0 ± 6.4	133.9 ± 8.2	148.1 ± 9.2	159.0 ± 9.1	168.6 ± 8.1	176.7 ± 9.2
VI	26	Female	111.9 ± 7.3	126.8 ± 8.6	139.0 ± 8.5	144.9 ± 11.4	156.2 ± 9.6	159.4 ± 12.7
VII	13	Female	104.3 ± 5.3	120.0 ± 7.3	135.1 ± 5.8	135.7 ± 5.8	153.3 ± 8.1	155.8 ± 11.2
VIII	10	Female	114.9 ± 8.1	120.9 ± 8.0	138.4 ± 9.4	149.9 ± 10.7	149.8 ± 11.3	171.0 ± 11.3
V	19	Male	148.5 ± 8.2	184.3 ± 12.0	219.3 ± 14.0	243.7 ± 18.2	266.1 ± 19.0	285.6 ± 20.5
VI	25	Male	144.3 ± 14.2	174.8 ± 17.9	203.3 ± 20.2	227.3 ± 22.4	237.9 ± 18.5	248.4 ± 18.0
VII	13	Male	131.6 ± 16.7	158.6 ± 15.8	188.5 ± 20.8	198.5 ± 23.6	212.0 ± 16.7	227.9 ± 18.8
VIII	15	Male	144.4 ± 14.9	171.6 ± 17.9	210.1 ± 16.3	224.0 ± 14.2	236.8 ± 13.7	266.9 ± 14.3

TABLE 8. Hemoglobin Values of 80-day-old Rats Maintained on Commercial Rations With Varying Levels of Molybdenum (grams per 100 milliliters of blood)

Animal No.	Group V	Group VI	Group VII	Group VIII
1	13.6	11.5	13.2	15.0
2	14.3	11.8	13.0	13.5
3	14.3	13.1	14.0	15.0
4	13.6	14.1	10.8	14.2
5	12.2	12.0	14.0	16.1
6	14.1	12.0	12.4	13.6
7	14.7	13.8	12.4	13.5
8	16.0	14.8	12.2	9.8
9	14.3	13.4	12.6	12.2
10	15.6	13.1	13.5	13.2
11	13.2	11.3	13.0	15.4
12	--	12.1	12.6	14.0
13	--	10.8	14.3	15.2
14	--	12.8	13.8	12.2
15	--	14.5	12.8	12.1
16	--	13.8	12.8	11.8
17	--	12.6	12.4	13.8
18	--	12.5	12.1	14.0
19	--	14.2	--	13.8
20	--	12.8	--	14.2
21	--	16.0	--	13.5
22	--	16.0	--	15.2
23	--	13.0	--	12.3
24	--	14.0	--	--
25	--	11.0	--	--
26	--	10.8	--	--
Average Weight and Deviation	14.2 ± 0.7	13.1 ± 0.9	12.9 ± 0.6	13.6 ± 1.1

were sacrificed at the end of the experiment and the livers and the femur, tibia and fibula bones were analyzed. The molybdenum and copper values of the livers and the molybdenum content of the bones are given in Table 9.

Discussion and Conclusions

The data obtained from a growth study with rats on the effect of high levels of molybdenum added to the commercial feed must be carefully studied to determine its full significance. Female animals receiving 160 parts per million of added molybdenum and an average level of 43.6 parts per million of copper (Group VIII), from weaning age (21 days) to 78 days of age had an average final weight of 171.0 grams, in comparison to 176.7 grams for the control animals (Group V), which received the normal ration containing an average of 31.5 parts per million of copper. The animals of Group VI, which received a level of 80 parts per million of added molybdenum and an average copper level of 35.6 parts per million, had a slightly lower average final weight of 159.4 grams. This difference is not great enough to be of significant importance. The females in Group VII, which received 120 parts per million of molybdenum and an average copper level of 32.7 parts per million, had an average final weight of 155.8 grams. There seems to be a tendency for high levels of molybdenum to decrease to a slight degree the final average weight of the rats at 78 days of age. When there was a slightly higher copper level, these rats grew as well as the control animals despite an increase in the molybdenum content of the ration (Group VIII). The males of the various groups show the same tendency. In Group V, the final average weight of 285.6 grams is comparable to the 266.9 grams

TABLE 9. Mineral Accumulation in the Liver and Bone of Animals Maintained on Commercial Rations Supplemented with Varying Levels of Molybdenum (expressed as parts per million)

Group	Animal No.	Copper in the Liver	Molybdenum in the Liver	Molybdenum in the Bone
V - Control	1	20.4	2.0	1.5
	2	21.0	2.2	1.1
	3	19.7	2.0	0.4
	4	21.8	2.4	0
	5	12.7	2.8	3.3
	6	25.3	2.1	--
VI - 80 parts per million of Molybdenum	1	20.3	3.2	4.3
	2	21.4	3.7	7.5
	3	19.7	4.7	6.2
	4	45.8	4.0	8.2
	5	14.3	4.3	--
	6	9.7	3.9	5.7
	7	18.3	4.7	12.1
	8	30.5	2.4	8.5
	9	14.0	2.2	4.3
	10	21.0	4.1	2.5
	11	23.8	1.4	13.4
	12	16.3	4.0	7.8
	13	28.0	3.9	2.3
	14	11.6	4.2	6.2
	15	14.1	4.5	--
	16	19.5	3.0	--
	17	14.0	2.7	10.4
	18	15.9	4.5	7.3
VII - 120 parts per million of Molybdenum	1	13.8	5.3	4.7
	2	17.6	5.7	7.7
	3	15.1	4.8	10.1
	4	27.4	5.3	5.6
	5	11.4	4.1	6.6
	6	21.3	2.5	13.8
	7	15.5	4.2	7.2
	8	10.3	5.1	11.6
VIII - 160 parts per million of Molybdenum	1	21.4	4.3	9.3
	2	27.4	6.0	14.8
	3	24.2	4.9	8.3
	4	33.8	4.3	10.9
	5	15.0	3.0	12.4
	6	14.9	4.4	1.6
	7	21.1	4.3	8.8
	8	20.9	4.9	9.8

for Group VIII, whereas Group VI had a lower weight of 248.4 grams and Group VII 227.9 grams. Due to climatic conditions which prevented the preparation of large quantities of ground feed, the mixed feeds were prepared weekly and shipments of the commercial feed were obtained from the retailer as required. The experimental trials with animals of Groups V, VI, and VII were almost completed before Group VIII was started. This accounts for the difference in the copper level of the mixed feed of this ration in comparison to the similar levels in the other rations.

The average hemoglobin values of the animals on the rations of this experiment at 80 days of age were 14.2, 13.1, 12.9 and 13.6 for Groups V, VI, VII and VIII, respectively. There appears to be only a slight, if any, effect as a result of the molybdenum level of the ration.

The copper level of the liver was approximately the same in all groups, although there were isolated cases of low values in all groups. There was an increase in the molybdenum content of the livers of the animals on supplemented levels of molybdenum. The increase was moderate with values ranging up to 7.7 parts per million, in comparison to an average of 2.3 in the controls. The accumulation of molybdenum in the bone of the animals on the high levels of molybdenum varied from 1.6 to 14.8 parts per million and was not constant in individual groups. The control animals show an average accumulation of 1.0 part per million.

The data obtained from this experiment has indicated that copper has a therapeutic value in overcoming, in part, the effects of molybdenum toxicity.

EXPERIMENT 3

Experimental Procedure and Results

In order to study the possible relation between the graying induced in the growing rat by copper deficiency and that by pantothenic acid deficiency, 18 rats were maintained on simplified Ration IV (see Table 1), a low copper-low molybdenum ration, from 21 days of age until they were 60 to 90 days of age. These animals were completely gray, as illustrated by Figure 4. The animals were divided into groups of three each and given various levels of calcium pantothenate and copper as indicated in Table 10.

TABLE 10. Treatments and Rations Given Copper Deficient Rats

Group	No. of Rats	Treatment	Ration
I	3	Control	IV (Low Copper-Low Molybdenum)
II	3	10 micrograms Calcium Pantothenate	IV (Low Copper-Low Molybdenum)
III	3	20 micrograms Calcium Pantothenate	IV (Low Copper-Low Molybdenum)
IV	3	30 micrograms Calcium Pantothenate	IV (Low Copper-Low Molybdenum)
V	3	40 micrograms Calcium Pantothenate	IV (Low Copper-Low Molybdenum)
VI	3	24 parts per million Copper	II (High Copper-Low Molybdenum)

The calcium pantothenate supplement was administered orally by stomach tube. At the end of five weeks, the haircoats of the animals receiving 10 micrograms were unchanged; however, those animals receiving 20 micrograms showed some response in repigmentation, whereas those animals

receiving 30 and 40 micrograms (see Figures 6 and 7) showed a pronounced repigmentation. The repigmentation proceeded in a regular pattern as illustrated in Figures 6 and 7. A dark stripe appeared on the lower part of the side and gradually increased in width. Figure 8 shows the contrast between a control animal and the response of a copper deficient animal to the vitamin after five weeks of supplementation. At the end of three months one animal receiving 40 micrograms was almost normal in pigmentation, but there was little change in the degree of repigmentation in the animals on lower levels of calcium pantothenate. There was no spontaneous recovery in the control animals. The animals in Group VI on a copper level of 24 parts per million exhibited almost complete repigmentation at the end of six weeks. The pattern was the same as in Groups III, IV, and V.

Both rations used in this experiment contained approximately 125 micrograms of pantothenic acid per 10.0 grams (83). Chemical analysis of the vitamin supplement has shown that less than 0.1 microgram of copper is present in the daily dose. Ration IV contained one part per million of copper and less than one part per million of molybdenum, whereas Ration II contained 24 parts per million of copper and less than one part per million of molybdenum.

Discussion and Conclusions

The graying of the haircoat of rats maintained on a low copper ration has shown itself to be related to a pantothenic acid deficiency. At the end of five weeks of oral administration of calcium pantothenate

FIGURE 6. Response of Pantothenic Acid on the Graying Produced by Copper Deficiency

FIGURE 7. Response of Pantothenic Acid on the Gray Copper Deficient Rat

FIGURE 8. Comparison of a Typical Copper Deficient Rat (Left) and An Animal Showing Response to Pantothenic Acid

supplements to gray copper deficient rats, the results have indicated that a 10-microgram daily dose has no effect on the haircoat of the animals, a 20-microgram daily dose exerts some effect on repigmentation, whereas doses of 30 and 40 micrograms cause a pronounced effect on the restoration of color to the haircoat of the lower part of the body (Figures 7 and 8). The complete restoration of color after three months of supplementation was obtained in one animal on a 40-microgram level. There was little change in the animals on lower levels. The same effect in repigmentation was obtained by placing the copper deficient rat on a copper level of 24 parts per million. The fact that there was no spontaneous recovery of pigmentation in the animals maintained on a low copper level and that there was an insignificant amount of copper in the daily dose indicates that the factor responsible for repigmentation in Groups IV and V was the calcium pantothenate supplement.

The results of this experiment have indicated that there is a relationship between the graying produced in rats by a copper deficiency and the pantothenic acid requirement. It can be postulated that copper may be related to pantothenic acid in some unknown enzyme system.

EXPERIMENT 4

Experimental Procedure and Results

The distribution of labeled copper, its excretion, and its absorption and retention in the blood has been studied in order to furnish data and information concerning the fate of copper in the bovine.

Five young calves and a young bull were given radioactive copper orally, in the form of copper nitrate, and sacrificed after approximately 42 hours. Animal 6 was given seven grams of sodium molybdate during the week prior to the dosage of labeled copper. The other animals did not receive any previous treatment. Representative samples were taken from the tissues, organs, and contents of the animals, and the percent of the dose in the sample and the concentration was determined on a relative basis (Table 11).

The excretion of labeled copper administered to the bovine was studied with four animals of approximately the same weight. The radioactive copper was introduced into two of the animals by injection into the jugular vein, one animal by intramuscular injection in the region of the neck, and the other by oral administration. Because of the short half-life of the isotope, the excretion studies were limited to 50 hours. The distribution of the dosage in the urine and the feces during this period is given in Table 12. The percentage figures given in the table are the percent of total dose which was excreted since the previous collection.

The percentage of labeled copper absorbed or retained in the blood and its concentration in micrograms per 100 milliliters of blood as

TABLE 11. The Distribution of Labeled Copper in the Bovine

Number of Animal	1	2	3
Age and Weight, in pounds	1 month - 60	8 days - 51	1½ months - 85
Mode of Administration	Oral	Oral	Oral
Actual Dosage, Mg. Copper	171	167	146
Sacrificed After, Hours	48	42	42

	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue
Pituitary	*	—	*	—	*	—
Thyroid	26	0.0027	23	0.0012	*	—
Thymus	23	0.0198	30	0.018	32	0.0320
Adrenals	*	—	63	0.0019	48	0.0015
Reproductive Organs	46	0.0031	29	0.0025	5	0.0017
Cerebrum	8	0.0107	6	0.0029	*	—
Cerebellum	9	0.0051	4	0.006	33	0.0080
Eye	6	0.0016	6	0.0023	*	—
Intestinal Lymph Glands	31	—	17	—	*	—
Heart	67	0.1340	7	0.013	8	0.019
Blood	24	0.5000	23	0.350	10	0.29
Aorta	26	0.0104	31	0.0091	*	—
Lung	26	0.0951	18	0.6600	8	0.37
Trachea	41	0.0148	20	0.013	7	0.0031
Kidney	220	0.2640	100	0.110	71	0.100
Bladder	28	0.0102	9	0.0023	4	0.0013
Bladder Urine	347	0.2350	—	—	69	0.0066
Tongue	23	0.0288	12	0.017	6	0.011
Esophagus	257	0.0931	28	0.0087	3	0.0012
Fundus Abomasum, Mucosa	1150)	0.1824	71)	0.090	42)	0.042
Fundus Abomasum, Muscular	345)		29)		13)	
Pyloric Abomasum, Mucosa	984)	0.0718	45)	0.013	44)	
Pyloric Abomasum, Muscular	254)		24)		9)	
Abomasum Contents	1741	2.6630	617	3.100	100	0.12
Reticulum	776	0.2480	35	0.011	17	0.011
Omasum	484	0.1970	51	0.022	100	0.075

TABLE 11. (Cont'd) The Distribution of Labeled Copper in the Bovine

Number of Animal	1		2		3	
	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue
Omasum Contents	--	--	--	--	690	0.38
Rumen	583	0.272	33	0.032	13	0.033
Rumen and Reticulum Contents	5112	16.511	1523	5.500	310	7.8
Duodenum, Mucosa	159	--	89)		•)	
Duodenum, Muscular	88	--	50)		10)	
Jejunum, Mucosa	103)	0.286	57)	0.23	31)	0.38
Jejunum, Muscular	49)		26)		16)	
Ileum, Mucosa	14	--	59)		32)	
Ileum, Muscular	50	--	32)		14)	
Small Intestine Contents	660	2.358	40	0.11	50	0.210
Large Intestine, Mucosa	146)	0.265	202)	0.250	45)	0.10
Large Intestine, Muscular	46)		34)		13)	
Large Intestine Contents	10936	13.342	1079	0.76	310	0.67
Pancreas	49	0.0199	68	0.023	17	0.0074
Spleen	33	0.0271	16	0.010	9	0.012
Liver	1572	10.51	835	4.50	1090	7.0
Gall Bladder and Bile	18	0.0004	229	0.011	97	0.0056
Tenderloin Muscle	13	--	7	--	6	--
Gastrocnemius Muscle	6	--	5	--	•	--
Ligament	22	--	19	--	•	--
Cartilage	--	--	51	--	•	--
Bone	•	--	15	--	•	--
Red Bone Marrow (Ribs)	109	--	92	--	15	--
White Bone Marrow (Femur)	105	--	19	--	9	--
Teeth	•	--	45	--	•	--
Hide	5	0.161	86	0.62	5	--

TABLE 11. (Cont'd) The Distribution of Labeled Copper in the Bovine

Number of Animal	4	5	6**
Age and Weight, in pounds	3 months - 127	18 months - 450	3 months - 160
Mode of Administration	Oral	Oral	Oral
Actual Dosage, Mg. Copper	137	256	137
Sacrificed After, Hours	42	42	42

	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue
Pituitary	*	—	*	—	*	—
Thyroid	*	—	*	—	*	—
Thymus	3	0.0053	3	0.0067	9	0.019
Adrenals	*	—	3	0.0005	*	—
Reproductive Organs	5	0.0028	1	0.0058	*	—
Cerebrum	*	—	*	—	*	—
Cerebellum	4	0.0086	*	—	*	—
Eye	*	—	*	—	*	—
Intestinal Lymph Glands	7	—	3	—	*	—
Heart	3	0.0107	1	0.0115	*	—
Blood	2	0.0388	2	0.121	6	0.1984
Aorta	2	0.0009	3	0.0019	*	—
Lung	4	0.0286	1	0.0224	—	—
Trachea	3	0.0019	*	—	15	0.0422
Kidney	15	0.0268	9	0.0446	10	0.0040
Bladder	*	—	1	0.0007	13	0.0047
Bladder Urine	2	0.0019	17	0.0396	*	—
Tongue	*	—	2	0.0147	*	—
Esophagus	6	0.0044	2	0.0059	*	—
Fundus Abomasum, Mucosa	23)		6)		*	—
Fundus Abomasum, Muscular	6)		3)		*	—
Pyloric Abomasum, Mucosa	23)	0.0449	11)	0.032	*	—
Pyloric Abomasum, Muscular	12)		3)		*	—
Abomasum Contents	89	1.911	39	0.573	50	0.4527
Reticulum	5	0.0076	4	0.0249	*	—
Omasum	14	0.0171	3	0.0457	11	0.0487

TABLE 11. (Cont'd) The Distribution of Labeled Copper in the Bovine

Number of Animal	4		5		6**	
	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue
Omasum Contents	545	0.2453	61	1.99	57	0.3593
Rumen	8	0.0584	2	0.0554	6	0.792
Rumen and Reticulum Contents	326	28.131	40	11.0	33	3.4711
Duodenum, Mucosa	13	—	10)		*	—
Duodenum, Muscular	6	—	*)		*	—
Jejunum, Mucosa	24	—	4)	0.097	13	—
Jejunum, Muscular	6	—	3)		13	—
Ileum, Mucosa	14	—	4)		*	—
Ileum, Muscular	5	—	58)		*	—
Small Intestine Contents	85	1.16	70	2.15	*	—
Large Intestine, Mucosa	10	—	8)	0.143	10)	0.1368
Large Intestine, Muscular	4	—	4)		8)	
Large Intestine Contents	691	3.137	755	29.1	*	—
Pancreas	16	0.0008	2	0.0024	*	—
Spleen	2	0.0030	3	0.0149	11	0.0181
Liver	55	0.4863	41	1.01	69	0.8164
Gall Bladder and Bile	*	—	2	0.0036	*	—
Tenderloin Muscle	*	—	1	—	*	—
Gastrocnemius Muscle	—	—	*	—	*	—
Ligament	3	—	1	—	*	—
Cartilage	—	—	*	—	*	—
Bone	—	—	*	—	*	—
Red Bone Marrow (Ribs)	—	—	2	—	*	—
White Bone Marrow (Femur)	—	—	*	—	*	—
Teeth	—	—	13	—	*	—
Hide	3	0.1217	1	0.228	*	—

* No detectable activity

** Given 7 gm. sodium molybdate during week prior to labeled dose

TABLE 12. Excretion of Labeled Copper Administered to the Bovine (Expressed as Percent of Administered Dose)

Number of Animal	1		2		3		4	
Age and Weight, in pounds	14 months - 300		14 months - 345		16 months - 345		16 months - 335	
Mode of Administration	Oral		Jugular Injection		Jugular Injection		Subcutaneous	
Dosage, Mg. Copper	253		131		145		145	
Time (Hours)	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine
19	23.01	0.212	0.524	0.810	0.78	1.04	0.19	0.86
26	12.08	0.148	0.215	0.620	0.40	0.18	0.42	0.20
43	13.48	0.360	0.493	—	0.68	0.60	1.04	0.33
50	4.11	0.098	0.190	—	0.21	0.30	0.38	0.76
Total	52.68	0.818	1.422	1.430	2.50	2.12	2.03	2.15

determined in five animals is presented in Table 13.

Discussion and Conclusions

The tissue distribution of labeled copper administered orally to five young calves and an 18-month-old bull has been reported in Table 11. It is evident that the absorption or retention of the total dose in the tissues is small. There is considerable variance in the relative concentrations of similar tissues and samples taken from the different animals of the experiment. The liver contained the highest accumulation of copper of the organs analyzed and the highest percent of the total dose present in the samples, with the exception of some of the gastro-intestinal contents. It is evident that the liver is the principle storage organ for copper. There was a high concentration of radioactive copper in the blood, kidney, rumen, lungs, small intestine composite, and large intestine composite. In two animals, there were high levels of the copper present in the hide and moderate levels in the red bone marrow. In the bladder urine, omasum, fundus and abomasum composite, heart, and reticulum of the other animals there was a moderate concentration of labeled copper. There was a low accumulation in the spleen, tongue, cerebellum, adrenals, and pancreas. The other samples analyzed contained extremely low concentrations or no detectable quantity. It is possible that some of these results may be due, in part, to the small sample available. The concentration of labeled copper expressed as micrograms per 100 grams of fresh tissue per 100 milligrams of dose in the mucosa of the various parts of the gastro-intestinal tract is relatively higher than that of the

TABLE 13. Blood Study of Cattle Administered Labeled Copper

	1		2		3	
Number of Animal						
Age and Weight, in pounds	14 months - 345		14 months - 345		14 months - 345	
Mode of Administration	Subcutaneous Injection		Jugular Injection		Jugular Injection	
Actual Dosage, Mg. Copper	145		145		131	
Time	Percent of Dose in Blood	Micrograms Per 100 ml.	Percent of Dose in Blood	Micrograms Per 100 ml.	Percent of Dose in Blood	Micrograms Per 100 ml.
5 Minutes	0.65	18	36.39	460	—	—
1 Hour	2.31	32	36.18	212	49.49	648
3 Hours	2.19	27	19.02	36	—	—
6 Hours	2.42	30	13.26	33	—	—
19 Hours	2.13	30	9.12	19	—	—
24 Hours	—	—	—	—	4.62	8
26 Hours	2.25	35	6.93	18	—	—
45 Hours	3.70	—	5.58	—	—	—
50 Hours	3.74	—	5.70	—	—	—
67.5 Hours	4.03	—	5.33	—	—	—

	4		5	
Number of Animal				
Age and Weight, in pounds	1 month - 60		2 months - 85	
Mode of Administration	Oral		Oral	
Actual Dosage, Mg. Copper	171		146	
Time	Percent of Dose in Blood	Micrograms Per 100 ml.	Percent of Dose in Blood	Micrograms Per 100 ml.
19 Hours	—	—	0.29	20
24 Hours	0.50	36	—	—

muscular portion of the tissues. The liver contained the highest accumulation of labeled copper and the kidney generally had a relatively high accumulation. The rumen and reticulum contents contained extremely high levels of the labeled copper. The large intestine contents of two animals were exceptionally high, although in all cases there was considerable activity present.

Animal 6 received seven grams of sodium molybdate during the week previous to the administration of the labeled copper. In many of the tissues in which activity was found in the other animals, there was no detectable copper. The contents of the gastro-intestinal tract contained little copper, if any, when the animal was sacrificed. The liver contained a normal concentration of labeled copper in relation to that obtained for Animal 4, a young calf of the same age, and Animal 5, a young bull.

The high percentage of the dose present in the tissues of Animals 1, 2 and 3 was due in part to the age and ration of the animals. Animals 1 and 2 were receiving milk and Animal 3 milk and calf starter feed, whereas Animals 4, 5 and 6 received hay, corn, cottonseed meal and bonemeal. The amount of undigested food present in the animal body will affect absorption of an administered supplemental dose.

Table 12 indicates that the greatest elimination of orally administered labeled copper occurs in approximately 19 hours. The elimination of labeled copper administered by jugular injection or subcutaneous injection does not reach a definite peak within 50 hours after administration. The elimination of 52.68 percent of the dose in the feces and 0.818 percent

in the urine of an animal orally administered, indicates poor absorption of the supplemental copper. Labeled copper administered by jugular injection or subcutaneous injection is eliminated in almost equal amounts in the feces and urine in a 50-hour experimental trial. During this period, there is approximately four percent of the dose eliminated. Almost one percent of the dose appears in the urine within 19 hours.

A study of the labeled copper present in the blood of the bovine is presented in Table 13. In Animal 2, when the labeled copper was administered by jugular injection, there was a rapid removal of copper from the blood within the first five minutes. There was 36.39 percent of the dose present in the blood five minutes after dosage and 36.18 percent after one hour. In Animal 3, there was 49.49 percent of the labeled copper in the bloodstream at the end of one hour. After an hour there was a gradual decline in the concentration in Animal 2. In Animal 1, which was administered by subcutaneous injection, there was a slow increase in concentration of labeled copper in blood throughout the experiment. In two animals administered orally there was very little copper in the blood 19 to 24 hours after dosage. There appears to be an equilibrium in the blood when there is 18 to 36 micrograms of labeled copper present in 100 milliliters of whole blood. The rapid removal of copper from the bloodstream must be accounted for by deposition in the tissues, as the excretion data (Table 12) indicates that there is a slow removal of injected copper from the body.

EXPERIMENT 5

Experimental Procedure and Results

The effect of molybdenum and phosphorus on the distribution and excretion of ingested copper has been investigated with 36 rats selected from the stock colony.

The effect of molybdenum and phosphorus on the accumulation of ingested copper in select tissues of the rat was determined by a comparative study. Sixteen rats were fasted overnight, for approximately 16 hours, and then divided into four equal groups. Group I received 7.3 milligrams of labeled copper, Group II 7.3 milligrams of labeled copper and 40 milligrams of inert molybdenum, Group III 7.3 milligrams of labeled copper and 23 milligrams of inert phosphorus, and Group IV 5.4 milligrams of labeled copper, 24 milligrams of inert molybdenum, and 23 milligrams of inert phosphorus. The accumulation of the radioactive copper in select tissues of these animals is reported in Table 14.

The excretion of ingested copper was studied by the administration of labeled copper to 20 rats selected from Experiment 1. These animals were approximately 90 days old and represented animals on both high and low levels of copper and molybdenum. They were raised from 21 days of age on simplified rations (Table 1) until used in this experiment. The percent of the dose excreted by individual rats within each group was erratic and as a result were not tabulated. There was an average of 47.46 (± 8.46) percent of the dose eliminated in the five-day period following administration. There was 98.43 (± 1.03) percent of this total activity eliminated in the feces.

TABLE 14. Effect of Molybdenum and Phosphorus on the Accumulation of Ingested Copper in the Tissues of the Rat (Micrograms of Labeled Copper per Gram of Fresh Tissue, Based on 10 Mg. Dose)

Group	Dosage	No. of Rats	Liver	Blood	Lung	Spleen	Kidney	Muscle	Hide
I	7.3 mg. Copper	4	46 ± 6 (2.5)*	15 ± 1	2 ± 0.3	5 ± 3	13 ± 3	0.5 ± 0.3	0.2 ± 0.03
II	7.3 mg. Copper 40 mg. Molybdenum	4	18 ± 5 (1.2)	11 ± 2	4 ± 2	4 ± 2	8 ± 1	0.6 ± 0.4	0.3 ± 0.04
III	7.3 mg. Copper 23 mg. Phosphorus	4	9 ± 5 (0.5)	10 ± 3	3 ± 2	3 ± 1	5 ± 1	0.7 ± 0.6	0.4 ± 0.3
IV	5.4 mg. Copper 24 mg. Molybdenum 23 mg. Phosphorus	4	7 ± 0.8 (0.5)	13 ± 2	3 ± 0.4	3 ± 0.8	6 ± 1	0.7 ± 0.1	0.4 ± 0.2

* The mean value ± the mean deviation; values in parentheses represent percentage of dose in whole organ.

Discussion and Conclusions

Molybdenum administered simultaneously with labeled copper reduced the normal accumulation of ingested copper in the liver by 60 percent and in the kidney by 39 percent. Simultaneous dosage of phosphorus and labeled copper resulted in a reduction to approximately 20 percent of the normal accumulation in the liver and 33 percent in the kidney. Simultaneous administration of the three elements did not provide any further appreciable reduction in the accumulation. The treatments did not appreciably effect the concentration in the other tissues. When labeled copper was administered alone, 2.5 percent of the ingested copper was present in the liver, in comparison to 1.2 percent when molybdenum was also administered, and 0.5 percent when phosphorus was given simultaneously with the copper. When copper was administered alone, the highest accumulation of the ingested copper was found in the liver and in decreasing amounts in the blood, kidney, spleen, lung, muscle and hide. The percent of the total dose of labeled copper excreted by the rat within five days after dosage varies greatly among animals. The elimination of 47.46 ($\pm$ 8.46) percent of the dose by the animals of this experiment indicates that there is a rapid removal of the dose from the body. The presence of 98.43 ($\pm$ 1.03) percent of this activity in the feces indicates that there is a relatively low absorption of the administered dose.

EXPERIMENT 6

Experimental Procedure and Results

To determine the rate of uptake and the distribution of labeled copper when given orally or injected intravenously in the rabbit, eight animals were selected from the experimental colony. Six of the animals received oral administration of labeled copper by stomach tube and were sacrificed in pairs at 6, 18 and 42 hours after dosage. The other two animals were sacrificed six hours after receiving the radioactive copper by injection into the auricular vein of the ear. The distribution of labeled copper, expressed as the percent of the total dose present in various parts of the animal, is given in Table 15.

Discussion and Conclusions

The relative accumulation of labeled copper administered orally to the rabbit varies to a certain degree in all of the experimental animals. The greatest accumulation of the copper is found in descending order in the large intestine contents, stomach contents, and liver. There is apparently a low absorption of the administered copper and a high accumulation or storage of the absorbed copper in the liver. There is a relatively high concentration present in the small intestine, stomach, small intestine contents, and hide, while there is a relatively moderate concentration in the bladder, urine, blood, kidney and lungs of animals sacrificed after six hours. The distribution of labeled copper in the animals sacrificed 18 and 42 hours after dosage followed the same trend, with the exception that there was a high concentration of copper in the

TABLE 15. The Distribution of Labeled Copper Administered to the Rabbit
(Expressed as Percent of Total Administered Dose)

Number of Animal	1	2	3	4	5	6	7	8
Weight, in grams	1411	1330	2100	2160	1255	2230	1940	2300
Mode of Administration	Oral	Oral	Oral	Oral	Oral	Oral	Injection	Injection
Actual Dosage, Mg. Copper	31	31	15	15	46	50	8	4
Sacrificed After, Hours	6	6	18	19	42	42	6	6
Thyroid	0	0	0	0	0.0019	0.0016	0.0020	0
Thymus	0.0026	0.0020	0	0	0.0031	0.0012	0.0184	0.0990
Adrenals	0	0	0	0	0	—	0.0217	0.0045
Reproductive Organs	0.0037	0.0073	0.0056	0.0106	0.0033	0.0266	0.113	0.363
Cerebrum	0.0019	0	0	0.0021	0.0025	0.0012	0.0111	0.0043
Cerebellum	0	0	0.0007	0	0	0.0023	0	—
Eye	0.0028	0.0026	0.0010	0.0020	0.0019	0.0021	0.0202	0.0485
Heart	0.0065	0.0077	0.0040	0.0057	0.00748	0.0084	0.210	0.112
Blood	0.121	0.165	0.0491	0.0963	0.119	0.219	7.193	8.03
Lung	0.0330	0.487	0.0088	0.0178	0.194	0.0273	0.412	0.409
Kidney	0.0563	0.0920	0.0162	0.0453	0.0958	—	3.607	2.83
Bladder	0.0037	0.0043	0.0051	0.0019	0	0.0050	0.0310	0.124
Bladder Urine	0.123	0.0598	0.0058	—	0.294	1.416	1.152	—
Stomach	0.496	0.756	0.0490	0.104	0.128	0.219	0.241	0.340
Stomach Contents	6.275	31.167	2.621	10.64	17.725	9.990	0	0.104
Small Intestine	0.812	0.822	0.399	0.481	0.261	0.311	1.189	1.32
Small Intestine Contents	0.485	0.904	0.452	0.398	0.233	0.680	0.0234	0.309
Large Intestine	0.539	0.601	0.160	0.112	0.175	0.187	1.781	1.71
Large Intestine Contents	39.77	18.733	8.080	22.93	25.683	23.13	0.711	1.98
Pancreas	0	0.0018	0.0015	0.0001	0.0016	0	0.0418	0.0402
Spleen	0	0	0	0	0	0	0.0358	0.0578
Liver	1.387	3.034	0.309	0.458	2.967	3.42	21.60	16.48
Gall Bladder	0	0.0070)	0.0012	0	0	0.0027)	0.0224	0.0066
Bile	0.0038	0.0154)		0.143	0.0146	0.116)		0.034
Bone	0	0	0	—	—	—	0	—
Hide and Hair	0.132	0.216	0.117	0.214	0.218	0.0013	3.709	8.024

bladder urine of the animals sacrificed after 42 hours. The greatest concentration of labeled copper, six hours after being injected into the ear vein of the rabbit, was in the liver and in descending order in the blood, hide, kidney, large intestine and contents, small intestine, bladder urine, lungs, stomach, heart, and reproductive system. Approximately 19 percent of the injected dose was present in the liver. This indicates that the liver is vitally concerned with the removal of excess copper from the bloodstream. The kidneys contain approximately three percent of the dose. The accumulation of copper in the liver after six hours when the dose was administered orally was approximately two percent of the total dose, and 0.07⅘ percent in the kidney. It is quite evident that the liver and kidney are important organs for the removal of copper from the blood.

EXPERIMENT 7

Experimental Procedure and Results

The distribution of molybdenum in the tissues of the bovine, the accumulation in the liver, and the absorption and retention of the administered molybdenum in the blood, has been investigated by the administration of radioactive molybdenum, in the form of sodium molybdate, to several steers.

Two 14-month-old steers, weighing 380 pounds each, have been used to investigate the distribution of labeled molybdenum in the bovine. A comparison has been made of the distribution of the isotope when it is injected into the jugular vein and administered orally. One animal was given orally the equivalent of 7.0 grams of molybdenum trioxide, and sacrificed at the end of 12.5 days. The other animal was given the equivalent of 1.9 grams of molybdenum trioxide by jugular injection and was sacrificed at the end of 5 days. The percent of the dose present in various parts of the animals and its concentration, expressed as micrograms of the dose present in 100 grams of fresh tissue per 100 milligrams of dosage, are reported in Table 16.

The accumulation of labeled molybdenum in the liver of the bovine has been investigated in four animals. Three of these animals were administered radioactive molybdenum orally, whereas the other animal received the dosage by injection into the jugular vein. The age, weight, mode of administration, and actual dosage of each animal is given in Table 17. The dose is expressed as grams of molybdenum trioxide. The samples were obtained by the liver biopsy technique, with the exception

TABLE 16. The Distribution of Labeled Molybdenum Administered to Cattle

Number of Animal	1	2
Age and Weight, in pounds	14 months - 380	14 months - 380
Mode of Administration	Oral	Jugular Injection
Actual Dosage, Grams of MoO_3	7.0	1.9
Sacrificed After, Days	12.5	5

	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue
Pituitary	*	*	13.8	0.000089
Thyroid	*	*	4.5	0.00047
Thymus	0.42	0.00035	4.0	0.012
Adrenals	3.2	0.00041	34.1	0.00037
Penis	0.63	0.0013	9.9	0.02
Salivary Glands	0.38	0.00050	5.6	—
Parotid Glands	0.81	0.00085	6.6	—
Cerebrum	*	*	2.1	0.00077
Cerebellum	*	*	1.0	0.0027
Spinal Cord	—	—	1.4	—
Eye	1.28	0.00082	26.5	0.017
Intestinal Lymph Glands	6.45	--	23.2	—
Heart	*	*	3.6	0.033
Blood	0.50	0.066	11.1	1.5
Aorta	0.31	0.00024	5.7	0.0075
Lung	0.97	0.016	11.0	0.17
Trachea	0.16	0.00036	5.8	0.0097
Kidney	3.73	0.017	26.3	0.13
Bladder	6.1	0.0052	7.1	0.0054
Bladder Urine	0.86	0.00061	38.8	0.062
Tongue	0.24	0.0013	4.9	0.028

TABLE 16. (Cont'd) The Distribution of Labeled Molybdenum Administered to Cattle

Number of Animal	1		2	
	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue
Esophagus	0.40	0.00090	4.8	0.012
Fundus Abomasum, Mucosa	*)		5.2)	
Fundus Abomasum, Muscular	0.46)	0.0031	2.3)	0.037
Pyloric Abomasum, Mucosa	*)		6.2)	
Pyloric Abomasum, Muscular	0.35)		4.4)	
Abomasum Contents	0.59	0.0046	11.4	0.21
Reticulum	0.40	0.0019	7.6	0.036
Omasum	0.29	0.0033	6.1	0.074
Omasum Contents	0.47	0.010	1.7	0.27
Rumen	0.39	0.012	11.2	0.38
Rumen and Reticulum Contents	0.28	0.056	7.1	1.16
Duodenum, Mucosa	1.65)		7.8)	
Duodenum, Muscular	0.50)		3.4)	
Jejunum, Mucosa	2.25)	0.037	10.9)	0.30
Jejunum, Muscular	1.75)		6.5)	
Ileum, Mucosa	1.21)		13.3)	
Ileum, Muscular	1.18)		5.9)	
Small Intestine Contents	0.34	0.0074	7.9	0.30
Large Intestine, Mucosa	2.16)	0.013	11.6)	0.16
Large Intestine, Muscular	0.39)		7.4)	

TABLE 16. (Cont'd) The Distribution of Labeled Molybdenum Administered to Cattle

Number of Animal	1		2	
	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue
Large Intestine Contents	0.61	0.021	22.8	0.52
Pancreas	0.54	0.00087	6.6	0.012
Spleen	3.29	0.012	7.5	0.032
Liver	5.47	0.12	26.5	0.69
Gall Bladder	—	—	5.2	0.0012
Bile	0.34	0.00009	1.6	0.00082
Tenderloin Muscle	*	—	1.7	—
Gastrocnemius Muscle	0.20	—	3.8	—
Ligament	0.19	—	5.7	—
Cartilage	2.0	—	6.1	—
Long Bone (Femur)	6.8	—	19.5	—
Rib Bone	8.9	—	29.4	—
Vertebra	3.8	—	21.9	—
Periosteum	*	—	9.6	—
Red Bone Marrow	1.65	—	12.2	—
White Bone Marrow	0.92	—	1.3	—
Teeth	4.37	—	15.6	—
Hide	0.92	0.14	8.9	1.41
Collected Feces	—	34.2	—	11.0
Collected Urine	—	45.2	—	36.7

* No radioactivity detected in sample.

TABLE 17. The Accumulation of Labeled Molybdenum in the Liver of the Bovine

Number of Animal	1		2	
Age and Weight, in pounds	18 months - 380		18 months - 380	
Mode of Administration	Jugular Injection		Oral	
Actual Dosage, Grams of MoO_3	1.9		7.0	
Time	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue
19 Hours	—	—	23	0.532
24 Hours	44	1.134	—	—
96 Hours	27	0.691	—	—
140 Hours	—	—	6	0.134
12.5 Days	—	—	5	0.124

Number of Animal	3*		4	
Age and Weight, in pounds	9 months - 175		12 months - 335	
Mode of Administration	Oral		Oral	
Actual Dosage, Grams of MoO_3	4.6		5.0	
	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue
21 Hours	6	0.0834	12	0.257
41 Hours	21	0.321	23	0.514
64 Hours	20	0.306	19	0.420
117 Hours	—	—	16	0.352

* Given 4 grams copper sulfate as a drench 45 minutes prior to dosage.

of the final liver samples of the two animals which were sacrificed for the tissue distribution study. The percent of the total dose in the liver is calculated and the relative concentration is expressed as micrograms in 100 grams of fresh tissue per 100 milligrams of dose.

The absorption and retention of administered radioactive molybdenum in the blood of the bovine was studied by collecting blood samples from the jugular vein at definite intervals after the administration of the dose. The percent of the dose in the whole blood and the percent of the activity in the blood contributed by the plasma is tabulated in Table 18.

The excretion of labeled molybdenum has been studied with three animals. The dose was administered orally to two of the animals and injected into the jugular vein of the third animal. The age, weight, and percent of the total dose excreted daily in the urine and feces are reported in Table 19.

Discussion and Conclusions

Two steers have been used to study the distribution of orally and intravenously administered radioactive molybdenum. The data accumulated in Table 16 from these animals indicate the relative accumulation of molybdenum in the tissues. Five days after the injection of the dose into the jugular vein the highest accumulation was found in the whole blood. Expressing the percent of the total dose present in the blood as 1000, the relative accumulation in descending order is as follows: whole blood 1000, hide 940, reticulum and contents 773, liver 460, large intestine contents 347, rumen 253, jejunum composite 200, small intestine contents

TABLE 18. Absorption and Retention of Labeled Molybdenum in the Blood of the Bovine

Number of Animal	1		2	
Age and Weight, in pounds	18 months - 380		18 months - 380	
Mode of Administration	Jugular Injection		Oral	
Actual Dosage, Grams of MoO_3	1.9		7.0	
Time (Hours)	Percent in Whole Blood	Percent of Activity Contributed by Plasma	Percent in Whole Blood	Percent of Activity Contributed by Plasma
1	21.48	73	—	—
3	—	—	0.81	84
5	12.83	84	—	—
8	11.67	79	—	—
19	—	—	0.64	64
24	7.07	71	—	—
67	—	—	2.78	59
72	3.60	69	—	—
96	1.47	64	—	—
139	—	—	0.28	66
300	—	—	0.07	—

TABLE 18. (Cont'd) Absorption and Retention of Labeled Molybdenum in the Blood of the Bovine

Number of Animal	3*	4
Age and Weight, in pounds	9 months - 175	12 months - 335
Mode of Administration	Oral	Oral
Actual Dosage, Grams of MoO_3	4.6	5.0

Time (Hours)	Percent in Whole Blood	Percent of Activity Contributed by Plasma	Percent in Whole Blood	Percent of Activity Contributed by Plasma
21	0.80	38	0.60	87
46	1.36	51	2.91	23
70	1.58	85	2.56	78
100	0.78	80	1.53	63
141	0.38	—	0.68	—
196	0.27	—	0.19	—
300	—	—	0.07	74

*Given copper sulfate drench 45 minutes previous to dosage.

TABLE 19. Excretion Studies of Labeled Molybdenum Administered to Cattle
(Expressed as Percent of Dose Per Day)

Number of Animal	1		2		3**	
Age and Weight, in pounds	14 months - 380		14 months - 380		9 months - 175	
Mode of Administration	Jugular Injection		Oral		Oral	
Actual Dosage, Grams of MoO_3	1.9		7.0		4.6	
Time (Days)	Feces	Urine	Feces	Urine	Feces	Urine
1	0.71	16.98	1.26	4.83	3.21	0.46
2	1.95	5.09	9.87	12.65	13.08	2.34
3	3.39	5.84	9.13	10.92	19.88	7.76
4	2.72	5.17	6.10	7.50	11.84	4.67
5	2.18	3.57	5.14	5.26	4.01	2.75
6	—	—	1.06	1.30	0.81	1.84
7	—	—	0.68	1.20	1.41	0.75
8	—	—	0.33	0.52	*	*
9	—	—	0.20	0.40	*	*
10	—	—	0.14	0.44	*	*
11	—	—	0.16	0.16	0.49	0.14
12	—	—	0.10	0.03	0.18	0.17
13	—	—	0.06	0.01	0.68	0.67
Total for 5 Days	10.95	36.65	31.50	41.16	52.02	17.98
Total for 13 Days	—	—	34.23	45.22	55.59	21.55

* Insufficient sample
** Given drench of 4 grams copper sulfate 45 minutes prior to dose

200, omasum contents 180, abomasum contents 140, and large intestine composite 107. When the radioactive molybdenum was administered orally, the highest relative accumulation in the animal 12.5 days after dosage was as follows: hide 1000, liver 857, whole blood 471, reticulum and contents 373, jejunum composite 264, large intestine contents 150, kidney 121, lung 114, large intestine composite 93, and spleen 86.

The highest concentration, expressed as micrograms of molybdenum trioxide per 100 grams of fresh tissue, occurs in the ribs when molybdenum is administered orally, and in decreasing concentrations in the femur, intestinal glands, liver, vertebra, kidney, spleen, and adrenals. The highest accumulation when the dosage is administered by injection is found in the adrenals and in decreasing concentrations in the rib bone, liver, kidney, eye, vertebra, femur, and red bone marrow. The accumulation of molybdenum in the bone occurs at a rapid rate, as does that of phosphorus. The percent of molybdenum in the skeletal system, based on an average of the concentration of molybdenum in the various bones analyzed, can be calculated. When molybdenum was administered by injection, 3.737 percent of the total was present in the bone in five days, in comparison to 1.183 percent in 12.5 days, when the animal was orally administered.

There is a rapid elimination of administered molybdenum by the bovine. There was 47.60 percent of the total dose excreted within five days after the dose was administered by jugular injection, in comparison to 72.66 percent when it was administered orally without additional supplement, and 70.00 percent when a drench of copper sulfate was given 45 minutes before the dosage. The difference in the last two figures is not

significant. However, there was 43.35 percent of the excreted activity present in the feces when the dose was administered without previous copper treatment, in comparison to 74.31 percent in the feces when copper was given previous to the dose. Copper appears to reduce the absorption of administered molybdenum. Of the total activity excreted, 23.00 percent was present in the feces of the animal receiving the injected molybdenum.

The deposition of labeled molybdenum in the liver of the bovine appears to be the most rapid in the first 41 hours after dosage. Nevertheless, there is relatively small amounts of the total dose concentrated in the liver. After the peak is reached, there is apparently a gradual decrease in accumulation. In Animal 2, 0.124 percent of the dose was present in the liver 12.5 days after dosage. The highest percent of the dose was found in Animal 1, 24 hours after injection of the labeled molybdenum into the jugular vein. Animal 3 received a copper sulfate drench previous to the oral administration of labeled molybdenum. The data of the excretion study indicate that this animal absorbed less of the dose from the gastro-intestinal tract than Animal 2. There appears to be a slight inhibiting effect on the accumulation of molybdenum in the liver as a result of this treatment.

The removal of injected molybdenum from the blood is rapid. At the end of one hour there was 21.48 percent of the dose remaining in the blood, and after 96 hours only 1.47 percent. Approximately 70 percent of the activity was present in the plasma. When labeled molybdenum was administered orally to three animals, there was a varying rate of accumulation

in the blood of the individual animals. After approximately 12 days there was less than 0.1 percent of the total dose in the blood. When the animals were dosed orally, the peak of concentration of a single dose in the blood seemed to be reached within 46 to 70 hours after dosage. The plasma contributes approximately 70 percent of the activity.

EXPERIMENT 8

Experimental Procedure and Results

The effect of phosphorus on the excretion of ingested molybdenum has been investigated with a number of rats selected from the stock colony. Five animals were given an equivalent of 41 milligrams of labeled molybdenum trioxide, in the form of sodium molybdate, and 32 milligrams of inert phosphorus, in the form of di-sodium phosphate by oral administration. An equivalent of 42 milligrams of labeled molybdic oxide, without supplementation, was administered to four control animals. The percent of the total dose excreted and the percent present in the urine and in the feces are reported for each animal (Table 20). Figure 9 is a graphic representation of this data.

Discussion and Conclusions

In the rat, phosphorus apparently increases the absorption of molybdenum from the gastro-intestinal tract. In a 228-hour experimental trial, there was an average of 42.06 (± 10.12) percent of the total activity eliminated in the feces when molybdenum was administered without supplement, in comparison to 11.42 (± 4.12) percent when a phosphorus supplement was given simultaneously with the labeled molybdenum. Figure 9 illustrates graphically the effect of phosphorus on the excretion of molybdenum. Phosphorus did not appear to effect the amount of activity excreted during this period.

TABLE 20. Effect of Phosphorus on Excretion of Ingested Molybdenum by the Mature Rat

Dosage	Animal No.	Micrograms of MoO_3 in Dose	Time (Hours)	Percent of Total Dose Eliminated	Percent of Dose in Urine	Percent of Dose in Feces	Percent of Total Excretion in Urine	Percent of Total Excretion in Feces
Mo^{99} + P^{31}*	1	41,000	228	83.15	75.91	7.24	91.29	8.71
	2	41,000	228	66.17	61.32	4.85	92.67	7.33
	3	41,000	228	64.76	52.98	11.78	81.81	18.19
	4	41,000	228	83.51	76.90	6.61	92.08	7.92
	5	41,000	228	84.72	72.06	12.66	85.06	14.94
	Average			76.46	67.83	8.62	85.58	11.42
Mo^{99}	6	42,500	228	69.01	33.40	35.61	48.40	51.60
	7	42,500	228	73.09	54.00	19.04	73.95	26.05
	8	42,500	228	79.83	37.71	42.12	47.24	52.76
	9	42,500	228	69.19	43.01	26.18	62.17	37.83
	Average			72.79	42.03	30.74	57.94	42.06

* 0.023 gram Di-sodium phosphate

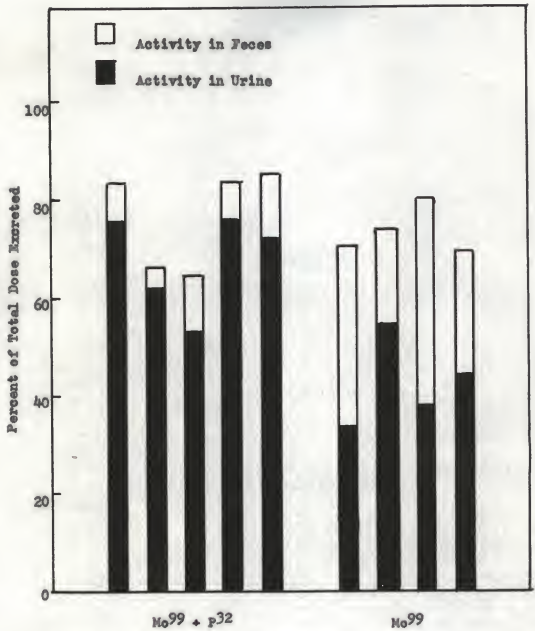

FIGURE 9. Graphic Representation of the Effect of Phosphorus on the Excretion of Ingested Molybdenum by the Rat During a 228-Hour Trial

EXPERIMENT 9

Experimental Procedure and Results

Radioactive phosphorus, administered orally to the bovine in the form of di-sodium phosphate, has been used to study the rate of absorption of administered phosphorus in the blood, its distribution in various parts of the animal, and its excretion.

The distribution of the isotope in the tissues, organs, and contents of a young five-month-old calf has been investigated. The animal was given a labeled solution containing 570 micrograms of phosphorus and sacrificed 118 hours after the administration of the isotope. The percent of the dose and the concentration of phosphorus in the various tissues, organs, and contents are reported in Table 21.

An excretion study of the rate of elimination of labeled phosphorus by the bovine has been studied with four young steers. The percent of elimination in the urine and feces is reported for a 16-day trial (Table 22). The absorption of phosphorus in the blood, expressed as percent of the total dose, and the actual dosage of each animal are reported during a 240-hour period (Table 23). The dosage was administered orally.

Discussion and Conclusions

The distribution of labeled phosphorus, administered orally to the young calf, indicates that the greatest accumulation of the dose, expressed as percent of the total dose, occurs in the bone after 118 hours. Approximately 8.70 percent of the total dose was present in the bone. The contents of the gastro-intestinal tract at the time of sacrifice contained 2.873

TABLE 21. Tissue Distribution of Labeled Phosphorus Administered to a Calf

Age and Weight, in pounds	5 months - 150
Mode of Administration	Oral
Actual Dosage, Micrograms	570
Sacrificed After, Hours	118

	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue
Pituitary	101	0.000667
Thyroid	5	0.0263
Thymus	75	0.0172
Adrenals	144	0.00947
Reproductive Tract	48	0.0667
Brain	18	0.0698
Submaxillary Gland	77	--
Parotid	139	0.0404
Intestinal Lymph Glands	148	--
Head Lymph	117	--
Heart	97	0.4280
Blood	9	0.490
Aorta	29	0.0170
Lung	81	0.5660
Trachea	114	0.1230
Kidney	137	0.3620
Kidney Fat	7	--
Bladder	74	0.0168
Bladder Urine	316	0.4600
Esophagus	74	0.0742
Eye	6	0.00315
Tongue	66	0.2030
Pyloric Abomasum	42	--
Pyloric Abomasum, Mucosa	175	--
Fundus Abomasum	53	--
Fundus Abomasum, Mucosa	90	--
Abomasum Contents	54	0.122
Reticulum	48	0.144

TABLE 21. (Cont'd) Tissue Distribution of Labeled Phosphorus Administered to a Calf

	Micrograms Per 100 Grams Fresh Weight Per 100 Mg. Dosage	Percent of Dose in Whole Tissue
Omasum	96	0.682
Omasum Contents	112	1.016
Rumen	46	0.585
Rumen and Reticulum Contents	58	4.18
Large Intestine	71	0.905
Large Intestine Contents	52	0.702
Duodenum Mucosa	157	—
Duodenum, Mucosa Removed	58	—
Ileum Mucosa	218	—
Ileum, Mucosa Removed	95	—
Jejunum Mucosa	159	1.473
Jejunum, Mucosa Removed	58	—
Small Intestine Contents	72	0.975
Pancreas	133	0.0982
Spleen	120	0.171
Liver	169	1.863
Gall Bladder	42	0.00505
Bile	24	0.00246
Tenderloin Muscle	55	—
Gastrocnemius Muscle	39	—
Ligament	6	—
Cartilage	22	—
Vertebra	175	—
Bone	122	8.70
Red Bone Marrow	217	—
White Bone Marrow	4	—
Teeth	107	—
Hide	19	0.886

TABLE 22. Excretion Studies of Labeled Phosphorus Administered to the Bovine (Expressed as Percent of Dose per Day)

Number of Animal	1		2		3		4	
Age and Weight, in pounds	12 months - 350		12 months - 345		8 months - 275		8 months - 225	
Mode of Administration	Oral		Oral		Oral		Oral	
Dosage, in Micrograms	100		100		7.5		7.5	
Time (Days)	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine
1	3.01	1.81	2.76	0.09	0	0.79	0	0.05
2	7.22	1.75	7.36	0.76	1.09	1.83	1.10	0.71
3	4.45	0.63	6.56	0.28	3.86	0.95	3.37	0.83
4	4.05	0.46	4.28	0.51	6.45	1.64	9.70	0.21
5	2.20	0.29	3.96	0.04	4.23	0.98	8.13	0.28
6	1.19	0.15	2.73	0.22	3.61	0.76	3.61	0.20
7	1.11	0.11	1.81	0.07	2.00	0.56	1.77	0.47
8	6.27	0.04	0.80	0.07	1.08	0.21	1.50	0.02
9	0.58	0.20	1.42	0.21	2.18	0.29	1.19	0.04
10	0.92	0.16	0.96	0.18	0.71	0.16	0.60	0.07
11	0.82	0.04	0.76	0.06	1.22	0.02	0.53	0.02
12	0.69	0.19	1.00	0.15	0.63	0.05	0.41	0.08
13	0.77	0.09	0.64	0.01	0.38	0.09	0.72	0.09
14	1.11	0.09	0.84	0.05	0.61	0.14	0.38	0.17
15	0.74	0.05	0.99	0.06	5.73	0.13	0.40	0.28
16	0.91	0.01	0.51	0.10	6.12	—	0.52	—
Total	36.04	6.07	37.38	2.86	39.90	8.60	33.93	3.52

TABLE 23. Accumulation of Labeled Phosphorus in the Blood of the Bovine When Administered Orally

Animal No.	Age in Months	Weight in Pounds	Actual Dosage in Microcuries	Percent of Dose in the Blood				
				24 hrs.	72 hrs.	120 hrs.	168 hrs.	240 hrs.
1	12	350	100	1.02	0.83	0.38	0.40	0.34
2	12	345	100	1.17	0.85	0.46	0.46	0.38
3	5	150	570	--	--	0.49	--	--

percent of the total dose, indicating the rapid elimination of the administered dose. There was a relatively high accumulation in the liver and decreasing amounts in the jejunum mucosa, large intestine, hide, lung, bladder urine, blood, heart, and kidney. Phosphorus, as would be expected, was found in every sample analyzed. On a unit weight basis, the highest concentration was found in the red bone marrow, and in decreasing concentration in the ileum mucosa, vertebra, pyloric abomasum mucosa, liver, jejunum mucosa, duodenum mucosa, intestinal lymph glands, adrenal glands, parotid, kidney, pancreas and bone.

The excretion of labeled phosphorus administered to the bovine did not seem to follow a definite pattern of elimination. The activity in the excreta was distributed in variable amounts throughout the duration of the experiment. However, at the end of 16 days there was an average of 36.81 (± 1.80) percent of the total dose eliminated in the feces and 5.26 (± 2.07) in the urine.

The concentration of labeled phosphorus, given orally to the bovine, was higher in the blood 24 hours after the administration of a single dose than it was at 72 hours or more. The percent of labeled phosphorus in the blood apparently reaches a comparatively constant level within 120 hours. The data indicate that this level was maintained for the duration of the experiment. There was an average of 0.44 (± 0.04) percent of the total dose in the blood 120 hours after administration.

EXPERIMENT 10

Experimental Procedure and Results

This experiment has investigated the effect of various factors on the absorption of phosphorus in the rat and the amount of administered dose remaining in the gastro-intestinal tract 24 hours after the administration of an oral dose.

The effect of molybdenum and copper on the accumulation of ingested phosphorus has been investigated with the mature six-month-old rat and the young 30 to 45-day-old animal. A number of rats from the stock colony were divided into seven groups. Groups I and V received only labeled phosphorus. In addition to the labeled phosphorus, in the form of di-sodium phosphate, Groups III and VII received simultaneously inert molybdenum, Group IV and VI inert molybdenum and copper, and Group I inert copper. The supplemental doses consisted of 5.18 milligrams of copper, in the form of copper sulfate, and 34.3 milligrams of molybdenum, in the form of sodium molybdate. These results are also comparative studies of the rate of absorption of the ingested phosphorus in the young and mature animal. Approximately 18 hours after dosage, the animals were sacrificed and the activity in select tissues was determined. The number of animals in each group, their treatment, and the accumulation of ingested phosphorus in various tissues are given in Table 24.

The effect of dietary levels of molybdenum on the accumulation of ingested labeled phosphorus in the blood, liver, kidney, and bone of the rat has been studied with a number of rats. The simplified Rations I (high molybdenum) and II (low molybdenum) of Experiment 1 (Table 1) were used

TABLE 24. Effect of Molybdenum and Copper on Accumulation of Ingested Phosphorus in the Tissues of the Rat (Micrograms x 10^4 Labeled Phosphorus per Gram of Fresh Tissue Based on One Microgram Dose per 100 Grams Body Weight)

Group No.	No. of Rats	Age	Treatment	Blood	Lung	Spleen	Liver	Kidney	Muscle	Bone	Hide
I	10	Mature	P^{32}	4 ± 1*	11 ± 2	13 ± 6	33 ± 10	20 ± 5	5 ± 1	20 ± 6	3 ± 1
II	10	Mature	P^{32} + Cu	4 ± 2	13 ± 6	19 ± 8	44 ± 17	23 ± 8	5 ± 2	22 ± 6	2 ± 0.6
III	9	Mature	P^{32} + Mo	6 ± 2	18 ± 7	24 ± 8	48 ± 6	32 ± 6	7 ± 3	46 ± 15	6 ± 2
IV	6	Mature	P^{32} + Mo + Cu	3 ± 1	8 ± 3	18 ± 6	29 ± 7	14 ± 5	5 ± 3	13 ± 3	3 ± 0.3
V	12	Young	P^{32}	13 ± 4	30 ± 9	63 ± 17	88 ± 18	66 ± 18	20 ± 5	260 ± 60	11 ± 3
VI	11	Young	P^{32} + Mo + Cu	9 ± 2	24 ± 7	55 ± 8	68 ± 17	50 ± 8	11 ± 4	133 ± 35	7 ± 3
VII	14	Young	Mo + P^{32}**	9 ± 2	28 ± 5	47 ± 15	53 ± 11	51 ± 11	17 ± 4	199 ± 38	8 ± 1

* The mean value ± the mean deviation.
** Rats given 22 mg. of molybdenum per day for 3 days preceding P^{32} dosage.

Labeled phosphorus dosage - 2.14 micrograms; additional copper - 5.18 mg.; additional molybdenum - 34.3 mg.

to raise 12 young rats, approximately 30 days old, for a period of 60 days. Half of these animals were fed Ration I and the others received Ration II. The animals were fasted overnight, for approximately 16 hours, before the oral administration of two micrograms of labeled phosphorus, in the form of di-sodium phosphate. Unfortunately, one animal on the high molybdenum ration had to be eliminated from the study as the dose was introduced into the lung rather than the stomach. The animals were sacrificed after 48 hours and the concentration and percent of ingested phosphorus in the blood, liver, kidney, and bone are reported in Table 25.

The effect on the accumulation of the phosphorus in the tissues, as a result of fasting rats before the administration of a labeled isotope, was considered as an important factor. The tissue distribution of labeled phosphorus, administered orally in the form of ortho-phosphoric acid, in 22 animals approximately 80 days of age is reported in Table 26. The animals were fed after weaning on Ration VI of Experiment 2, a commercial ration containing 80 parts per million of added molybdenum. These animals were divided into two groups, one of which was not fasted before receiving an oral dose of labeled phosphorus, and the other which was fasted for approximately 16 hours before the phosphorus was administered. Two of the animals which were fasted were eliminated because of difficulty encountered in dosing. The treatment given each group and the distribution of the labeled phosphorus in select tissues are given in Table 26.

TABLE 25. Effect of Dietary Molybdenum on Accumulation of Ingested Phosphorus in the Tissues of the Rat (Based on One Microgram of Dose per 100 Grams of Body Weight)

Treatment	Animal No.	Weight in Grams	Blood		Liver		Kidney		Bone
			Micrograms x 10^4 Per Gram of Fresh Tissue	Percent of Total Dose	Micrograms x 10^4 Per Gram of Fresh Tissue	Percent of Total Dose	Micrograms x 10^4 Per Gram of Fresh Tissue	Percent of Total Dose	Micrograms x 10^4 Per Gram of Fresh Tissue
Ration I	1	199	11	0.78	35	1.51	65	0.55	147
	2	186	13	0.91	115	4.35	74	0.60	183
	3	151	8	0.53	114	4.65	53	0.47	195
	4	167	8	0.58	144	4.90	100	0.70	258
	5	167	6	0.40	60	1.90	28	0.23	79
	Average	174	9 (± 2)	0.64 (± 0.16)	94 (± 37)	3.46 (± 1.39)	64 (± 17)	0.51 (± 0.13)	172 (± 47)
Ration II	6	172	33	2.34	177	6.45	101	0.75	240
	7	147	21	1.49	162	6.95	115	0.80	361
	8	182	13	0.89	145	5.70	77	0.50	334
	9	199	19	1.32	140	5.35	120	0.80	423
	10	208	21	1.46	161	6.35	123	0.75	255
	11	184	20	1.42	151	6.55	102	0.65	300
	Average	182	25 (± 6)	1.49 (± 0.34)	156 (± 11)	6.23 (± 0.47)	106 (± 13)	0.71 (± 0.10)	319 (± 52)

TABLE 26. Effect of Fasting and Non-Fasting Rats on the Accumulation of Ingested Phosphorus in Select Tissues (Micrograms x 10^5 Phosphorus per Gram of Fresh Tissue per 0.01 Microgram Dose per 100 Grams of Body Weight)

Treatment	Animal No.	Blood	Lung	Kidney	Spleen
Fasted	1	1.8	7.2	12.2	12.0
	2	2.6	7.2	14.9	11.5
	3	2.7	8.0	11.3	4.7
	4	2.0	6.1	10.9	10.8
	5	0.8	1.5	2.9	3.8
	6	2.2	6.3	10.3	11.0
	7	1.1	3.7	11.1	8.6
	8	1.2	3.1	1.5	5.5
	9	1.9	—	11.9	7.6
	Average	1.8 (± 0.5)	5.3 (± 1.9)	9.7 (± 3.3)	8.4 (± 2.7)
Not Fasted	1	1.5	1.9	8.3	6.2
	2	1.4	2.6	6.0	4.4
	3	—	3.3	3.5	5.6
	4	0.9	2.5	3.5	4.5
	5	0.9	2.5	3.3	4.5
	6	0.7	1.8	3.1	3.1
	7	0.8	1.2	3.4	3.1
	8	1.1	2.7	3.9	5.7
	9	0.9	3.0	5.1	4.9
	10	1.0	2.1	3.3	4.1
	11	0.4	2.2	1.3	—
	Average	1.1 (± 0.3)	2.3 (± 0.4)	4.1 (± 1.3)	4.6 (± 0.8)

TABLE 26. (Cont'd) Effect of Fasting and Non-Fasting Rats on the Accumulation of Ingested Phosphorus in Select Tissues (Micrograms x 10^5 Phosphorus per Gram of Fresh Tissue per 0.01 Microgram Dose per 100 Grams of Body Weight)

Treatment	Animal No.	Liver	Muscle	Bone	Hide
Fasted	1	19.8	3.6	34.4	2.1
	2	19.2	4.9	31.4	2.4
	3	18.4	2.8	34.0	1.4
	4	14.8	2.7	23.8	2.1
	5	4.5	1.2	11.0	0.6
	6	15.9	3.1	25.4	1.4
	7	10.9	0.6	17.3	2.8
	8	12.8	2.9	22.5	1.6
	9	16.4	4.2	33.8	2.4
	Average	14.7 (± 3.6)	2.9 (± 0.9)	26.0 (± 6.6)	1.9 (± 0.5)
Not Fasted	1	10.4	1.1	13.6	0.8
	2	9.1	1.3	12.4	0.9
	3	4.9	—	8.5	0.6
	4	5.0	1.6	10.6	0.8
	5	1.3	1.1	8.3	0.7
	6	3.8	1.1	6.6	0.7
	7	5.4	1.3	9.2	0.8
	8	7.0	1.2	17.9	0.6
	9	6.6	1.5	16.0	1.0
	10	8.6	2.4	17.5	1.1
	11	3.5	1.1	8.6	0.4
	Average	6.0 (± 2.2)	1.4 (± 0.3)	11.7 (± 2.3)	0.8 (± 0.1)

Discussion and Conclusions

The effect of molybdenum and copper on the accumulation of ingested phosphorus in select tissues of the rat is shown in Table 24. Copper administered simultaneously had little, if any, effect on the accumulation of labeled phosphorus. The simultaneous administration of molybdenum resulted in an increased accumulation of the phosphorus in the tissues. The values obtained in Group IV indicate that there is a tendency for simultaneous administration of copper and molybdenum to reduce the accumulation of phosphorus in the lung, liver, and kidney, and to increase the concentration in the spleen. The vast difference in the accumulation of ingested phosphorus in the tissues of the mature rat and the young rapidly growing rat is shown by comparing the values of Groups I and V. The data indicate that the young rat accumulated almost threefold the activity in the select tissues, with the exception of the bone, as did the mature animal. In the bone, the difference in accumulation was much more evident, being approximately tenfold. Group VI indicates that there is a tendency for copper and molybdenum to decrease the accumulation of phosphorus in the bone of the growing rat. There was approximately a 50 percent decrease in the normal accumulation. The values for the animals of Group VII indicate that the feeding of molybdenum previous to dosage tends to reduce the accumulation of phosphorus in the tissues.

Under normal conditions of dosage, the highest accumulation of phosphorus, expressed as a unit of fresh weight, in the select tissues of the mature rat occurs in the following decreasing order: liver, kidney, bone, spleen, lung, muscle, blood, and hide. In the young animal the relative

concentration in the tissues is the same, with the exception that there is an extremely high concentration in the bone.

The influence of dietary molybdenum on the absorption of phosphorus is emphasized in Table 25. Animals on a high molybdenum-high copper ration have exhibited a decidedly lower accumulation of phosphorus in the blood, liver, kidney, and bone. Both groups exhibit the highest accumulation in the bone, and decreasing amounts in the liver, kidney, and blood.

Apparently one of the factors influencing the accumulation of ingested phosphorus in select tissues of the rat is the amount of material present in the stomach at the time of dosage. When the experimental animals were fasted for approximately 16 hours previous to the administration of the dose, approximately a twofold accumulation was obtained in the tissues, in comparison to that obtained when the animals were not fasted. This can in part be explained by the dilution of labeled phosphorus by the substances present in the stomach. The amount of labeled phosphorus in the gastro-intestinal tract of the fasted and non-fasted animals indicates that 24 hours after the administration of labeled phosphorus, there was an average of 10.280 (± 3.027) of the total activity present. There was considerable variance in animals receiving similar treatment.

GENERAL DISCUSSION AND CONCLUSIONS

The Effect of Various Levels of Copper and Molybdenum on the Metabolism of the Rat: At the levels used in these experiments, the toxicity of molybdenum was dependent on the copper level of the ration. The toxicity is characterized in the rat by a severe diarrhea, rough haircoat, alopecia, poor development of the bones, loss of weight, degeneration of the liver, lacrimation of the eyes, and death. The severity of the symptoms was related to the degree of toxicity. The female animal appeared to be more susceptible than the male. The toxicity of a high level of molybdenum in the young animal deficient in copper was emphasized by the 100 percent mortality in the experimental animals. It has been demonstrated that increased levels of copper prevented most of the apparent symptoms of molybdenum toxicity. However, there was some indication of a retardation of growth. There was an increased storage of copper and molybdenum in the liver and of molybdenum in the bone when these elements were at high levels in the rations. The role of copper in preventing molybdenum toxicity has not been adequately explained. Neilands et al (142) have suggested that the toxicity of molybdenum may be neutralized to some extent by combination with copper, although the therapeutic dose of copper is insufficient to account for the formation of an insoluble copper molybdate.

The symptoms observed in molybdenum toxicity were in agreement with the observation of Britton and Goss (19), who reported that affected cattle were characterized by emaciation, changes in the haircoat, marked anemia, and a severe diarrhea. Beath and co-workers (8) reported that a high

level of molybdenum in the feed of livestock resulted in an erosion of the long bones and other pathological symptoms, which were not described. Neilands et al (142) reported that there were no gross abnormalities in rats maintained on high levels of molybdenum other than extreme emaciation. The lack of agreement with the results of this investigation may be due to the small number of animals used by Neilands and his co-workers and the very high level of copper in their basal ration. The severe diarrhea observed in the "molybdenum toxic" rats of this investigation corresponds with the scouring of cattle which has been traced to the molybdenum content of the forage (63, 65, 112, 113).

Copper deficiency in the rat is characterized by a marked anemia, graying of the haircoat, retarded growth, and a failure in reproduction. Evidence has been presented that high levels of molybdenum in a copper deficient ration do not result in an earlier appearance of the symptoms of copper deficiency in the rat. Neal et al (141) and Neal (140) have reported that copper deficiency in livestock resulted in anemia, a retardation of growth, and an impaired reproduction. The lack of abnormalities in the animals of this experiment maintained on a low copper ration is contrary to the reports of many investigators (36, 37, 191), who have worked with other species. Other workers (140, 141, 178) have reported that diarrhea is a characteristic symptom of copper deficiency. This symptom did not develop in the animals of this investigation which were maintained on a low copper-low molybdenum ration, but was apparent when there was a high level of molybdenum in the ration. The production of anemia by the lack of copper is in agreement with the work of many

investigators (170).

The graying of the hair of the black and piebald rat maintained on a copper deficient ration follows a characteristic pattern. The role of copper in pigmentation has not been adequately explained. In 1931 Cunningham (30) suggested the possibility that copper catalyzes the formation of melanin by the oxidation of "dopa" (l-3,4 dihydroxy-phenyl-alanine) which is present in the hair of the young growing animal. No confirmation of this theory has been found in the literature. The graying has responded to the administration of either copper or pantothenic acid. This indicates that there is a relationship and a possible accentuation of the requirement of pantothenic acid in copper deficiency. Henderson et al (85) reported that calcium pantothenate had no effect on the graying produced by copper deficiency. The composition of the ration used by these investigators was different from that reported here. There is no indication of the number of animals used by them nor the length of time that the supplements were given.

The Fate of Radioactive Phosphorus in the Animal Body: A study of the distribution of radioactive phosphorus administered orally to the bovine indicates that phosphorus is found in every tissue. The largest percent of the absorbed radioactive phosphorus was accumulated in the skeletal system. The greatest part of the bone activity is due to the accumulation of labeled phosphorus in the apatite structure (23, 27, 45, 87, 124). It has been reported that labeled phosphorus is stored primarily in the bone (88). In this investigation with the bovine, the liver contained a relatively high percent of activity and decreasing amounts were

found in the jejunum mucosa, large intestine, hide, lung, blood, heart, and kidney. The concentration, expressed on a unit weight basis, indicated that the highest concentration was in the red bone marrow, and relatively high concentrations were found in the various mucosae, intestinal lymph glands, adrenal glands, parotid, kidney, pancreas, and bone. In the mature rat the highest concentration, on a unit weight basis, was in decreasing order in the following tissues: liver, kidney, bone, spleen, lung, muscle, and hide. The same trend was noted in the young rat, with the exception that the highest accumulation was in the bone. The young rat showed a tendency to accumulate much larger quantities of activity in the tissues. It is natural that the need for phosphorus for rapid growth would tend to result in a greater absorption of dietary phosphorus by the young rat than the mature animal.

The results of these distribution studies are in general agreement with other investigators. Warren and Cowing (180) found that there was a material degree of absorption in the spleen, liver, kidney and bone. Couceiro (28) has reported that in periods up to 30 days after the injection of labeled phosphorus, the greatest absorption of phosphorus was in the bone, and that relatively small quantities were absorbed by the muscle, liver, and kidney. Cohn and Greenberg (26) found that based on a unit of fresh weight, phosphorus was retained in the select tissues according to the following decreasing order: bone, liver, kidney, lung, muscle, and skin.

The rat was used as the experimental animal to investigate the effect of copper and molybdenum on phosphorus deposition. In the mature

animal, copper given simultaneously with labeled phosphorus had little effect on the accumulation of phosphorus in the tissues. Molybdenum given simultaneously showed a tendency to increase the accumulation of phosphorus in the tissues, and copper and molybdenum together showed a tendency to reduce the accumulation in the lung, liver, kidney, and bone, and to increase the concentration in the spleen. This selective absorption in the tissues cannot be explained. The formation of an insoluble copper-phosphorus-molybdenum complex cannot be entirely discounted. The influence of molybdenum and copper in the young growing rat is definite, as there was approximately a 50 percent decrease in normal bone accumulation of labeled phosphorus when the elements were given simultaneously. Animals which were maintained on a high dietary level of molybdenum had a lower absorption of phosphorus in the blood, liver, kidney and bone than did animals on a normal ration. The failure to fast animals prior to dosage also resulted in lowered accumulation in the tissues. The effects of molybdenum and copper on phosphorus deposition in the bones of the young rat may be due to interference in the enzyme systems necessary for bone development.

The elimination of phosphorus by the bovine occurs principally in the feces. These results are in agreement with those reported by Hahn et al (79), who used the rabbit as the experimental animal.

The highest level of orally administered labeled phosphorus in the blood of the bovine occurred within the first 24 hours. A comparatively constant concentration was reached 120 hours after dosage.

The Fate of Radioactive Copper in the Animal Body: The use of labeled copper has provided a means of determining the fate of dietary and supplemental copper in the animal body. Experiments with the bovine, rat, and rabbit have shown that only a small proportion of orally administered labeled copper is absorbed. It is evident that the absorbed copper, like phosphorus, is widely distributed throughout the animal body. The consistently high accumulation of copper in the liver, blood, and kidney is of significance in view of the role of copper in hemoglobin formation. The spleen showed a relatively high accumulation in the rat, but not in the other species. The red bone marrow, which was only analyzed in experiments with the bovine, also exhibited a high level of accumulation. The hematopoietic function of these tissues has made the accumulation of copper important. It is apparent in the bovine that the young animal, which has a high copper content in the liver, continues for at least the first two months of life to accumulate copper rapidly. The high concentration of copper in the liver is in agreement with the reports of many investigators (30, 68, 84, 172). Schultze and Simmons (172) found that the greatest accumulation of labeled copper in the anemic rat occurred in the liver, kidney, and bone marrow. They determined the accumulation in a small number of select tissues. In this experiment, the high accumulation of copper in the hide is significant, as a result of the color changes which occur in copper deficiency, and may be important in the determination of the causes and the prevention of hyperkeratosis in cattle.

The importance of the liver as the principal organ of copper storage

is stressed by the relatively high accumulation of the activity absorbed by the experimental animals. This is emphasized by the rapid accumulation of approximately 19 percent of the total injected dose in the liver of the rabbit within six hours after administration. The kidney also functions in the rapid removal of excess copper from the bloodstream. The most rapid removal of injected copper from the bloodstream occurred within the first five minutes. The data indicate that this is accomplished primarily by deposition of the excess copper in the tissues as a result of the circulation in the blood system. The removal may also be due in part to the absorption by the red blood cells.

The poor absorption of copper in the bovine was emphasized by the elimination of over half the dose in the first 50 hours after its administration. The most rapid elimination occurred in the first 19 hours. When the copper was given by injection, approximately four percent of the dose was eliminated in the same period, this being equally divided in the feces and urine. In the first five days approximately 47 percent of the copper given orally to the rat was eliminated. Approximately 98.5 percent of the activity excreted by the bovine and the rat in these experimental trials was present in the feces. The results of this experiment are in agreement with those of other investigators. Eden (52) and Lindow et al (116) have reported that there was a high elimination of supplemental copper administered orally to the rabbit and rat. Houk et al (94) have reported that only 3.0 to 6.2 percent of the dietary copper was absorbed under varying conditions.

Simultaneous administration of molybdenum appeared to decrease

the accumulation of copper in many of the tissues of the bovine, with the exception of the liver. The effect on the accumulation of copper in the tissues of the rat appears to be different. Molybdenum reduced the accumulation of copper in the liver and kidney, but did not appear to effect the accumulation in other select tissues. Phosphorus, administered with labeled copper, induced a more startling decrease in the accumulation of copper in the liver and kidney, but like molybdenum did not appreciably effect the other select tissues. Molybdenum and phosphorus, administered with labeled copper, did not result in further reduction in the accumulation.

The Fate of Radioactive Molybdenum in the Animal Body: The distribution of orally administered and injected radioactive molybdenum in the bovine indicates that it is widely distributed in the body and that there is a similarity in its deposition and that of phosphorus. The deposition of 3.737 percent of the total injected dose in five days and 1.183 percent of the orally administered dose in 12.5 days in the skeletal system indicates that the greatest accumulation occurs in the bones. The ribs have a higher accumulation of molybdenum, expressed on a unit weight basis, than the femur or vertebra. There is a relatively high percentage of the absorbed dose present in the whole blood, hide, and liver. The animals receiving an oral dosage had a moderate accumulation in the kidney, lung, and spleen. The results indicate that the highest accumulation, on a unit weight basis, in the tissues of a bovine given labeled molybdenum orally occurs in decreasing order in the bone, intestinal lymph glands, liver, kidney, spleen, and adrenals. The results are not in strict

agreement with those of Neilands et al (142), who reported that in the rat there was slightly higher levels of radioactive molybdenum in the kidney and bone, on a unit weight basis, than in other select tissues. The difference in results is due, in all probability, to species differences, the extremely short duration of the investigation of Neilands and his co-workers, and the small number of select tissues analyzed by these investigators. Fairhall et al (62) reported that the greatest storage of inert molybdenum is in the kidney and bone.

When the labeled molybdenum was injected into the bovine, there was evidence of considerable activity in the various parts of the stomach, the intestinal tract, and their contents. It seems probable that the presence of this activity in the stomach must be accounted for by the passage of the injected molybdenum into the large quantities of saliva, which are secreted by the bovine, and its movement into the upper regions of the digestive system. The presence of activity in the intestines and their contents is in part due to the activity present in the upper regions of the digestive system and also to the activity which is probably present in the intestinal secretions, bile and pancreatic juices.

The excretion of 47.60 percent of the total injected dose in the first five days indicates that there is a rapid elimination of the dose by the bovine. This is further emphasized by the elimination of approximately 71 percent of the oral dose in a similar period by two other animals. Fairhall et al (62) have reported that the excretion and absorption of inert molybdenum are rapid. Copper sulfate, in this investigation, did not appreciably affect the total activity eliminated, but did increase the

percent in the feces. Copper appears to decrease the absorption of molybdenum. The data further indicate that copper results in a slight inhibiting effect on the accumulation of molybdenum in the liver and a decreased level in the blood. Simultaneous administration of phosphorus did not appear to affect the total amount of activity excreted by the rat during an experimental trial, but did increase the absorption from the intestinal tract. The data of Neilands et al (142) indicate that molybdenum is principally eliminated in the urine of the rat. This is in agreement with the experiments with the rat, but is contrary to the results of studies with the bovine.

The most rapid absorption of molybdenum by the liver occurs in the first 41 hours after dosage. There is, however, a small amount of the total dose accumulated in the liver. The removal of molybdenum from the blood of the injected animal is rapid during the first hour after injection. Approximately 78.52 percent of the dose was removed from the bloodstream during this period. The peak of molybdenum in the blood occurs within 46 to 70 hours after oral dosage. The plasma contributes 70 percent of the activity.

SUMMARY

The effects of levels of copper and molybdenum in the simplified rations (copper ranging from one to 34 parts per million, and molybdenum from less than one to 80 parts per million) and in the commercial rations (copper ranging from 31.4 to 43.6 parts per million, and molybdenum from 80 to 160 parts per million added) have been investigated with the rat. The relation between molybdenum toxicity and the dietary level of copper has been demonstrated. The following observations were made:

(1) Molybdenum toxicity is characterized by severe diarrhea, weakness, loss of weight, and a rough haircoat. It may also result in degeneration of the liver, a retarded skeletal development with poor calcification of the bone, a severe lacrimation, and death to the animal.

(2) In molybdenum toxicity, the indications are that mortality among the female rat is greater than among the male animals. There is evidence of high accumulation of molybdenum in the bone and liver.

(3) Copper deficiency is characterized by anemia, retarded growth, graying of the haircoat of black and piebald rats, a failure in reproduction, and a reduction in the level of copper in the liver.

(4) Copper exerts a therapeutic effect in overcoming, in part, the effects of high molybdenum levels in the ration.

(5) High levels of molybdenum (80 to 160 parts per million) in a high copper (31.4 to 43.6 parts per million) commercial ration did not appear to appreciably affect the hemoglobin value of the rat.

(6) The graying induced by copper deficiency in the black and piebald rat responded to supplements of pantothenic acid or copper. Copper

deficiency appears to accentuate the requirement of the rat for pantothenic acid.

The radioactive isotopes, Mo^{99}, P^{32} and Cu^{64}, have been used to determine the fate and interrelationships of these elements in the animal organism. The data indicate the following observations:

(1) The distribution of the elements in select tissues of experimental animals under normal and special conditions is demonstrated.

(2) The liver and kidney are the principal storage organs for copper. Phosphorus and molybdenum are primarily stored in the bone, although there are high accumulations in the liver. All three elements are widely distributed.

(3) There is a similarity in the accumulation of phosphorus and molybdenum in the tissues.

(4) Phosphorus and copper are apparently poorly absorbed. Molybdenum appears to be rapidly absorbed and eliminated. It is largely eliminated in the feces of the bovine and in the urine of the rat. Phosphorus and copper are principally eliminated in the feces. Approximately 98.5 percent of the orally administered copper eliminated by the rat and the bovine is in the feces. Intravenously injected copper is apparently retained in the body for a considerable time.

(5) The highest level of orally administered phosphorus in the blood of the bovine occurs in the first 24 hours and molybdenum in 46 to 70 hours. Injected copper is rapidly removed from the bloodstream in the first five minutes and molybdenum during the first hour.

(6) The liver and kidney are vitally concerned with the removal

of excess copper from the blood. The rabbit at the end of six hours accumulated approximately 19.0 percent of the total injected dose in the liver and 3.0 percent in the kidney.

(7) The young rat accumulated approximately threefold the activity of P^{32} in select tissues and tenfold in the bone, in comparison to that accumulated by the mature animal.

(8) There is a relatively high accumulation of activity in the digestive system of the bovine five days after the labeled molybdenum was injected into the jugular vein.

(9) Copper in the mature rat had little effect on phosphorus accumulation, molybdenum showed a tendency to increase accumulation in select tissues, and copper and molybdenum together reduced accumulation in the lung, liver, and kidney. In the young animal, molybdenum and copper resulted in approximately a 50 percent decrease in the accumulation of phosphorus in the bone. Animals maintained on a high dietary level of molybdenum had a lowered accumulation of P^{32} in the select tissues. Failure to fast animals before dosage decreased absorption.

(10) Molybdenum appears to decrease the accumulation of copper in many tissues of the bovine, with the exception of the liver; however, in the rat there is a reduction of accumulation in the liver and kidney. Phosphorus administered simultaneously causes an even greater decrease in these tissues.

(11) Copper did not affect the total activity of Mo^{99} eliminated by the bovine, but appeared to decrease absorption, inhibit accumulation in the liver, and decrease the level in the blood. Phosphorus did not

appear to affect total activity excreted by the rat; however, it increased the absorption of molybdenum.

The accumulation of molybdenum in the bone and liver of the young animal in experiments of longer duration may prove to be significant factors in health, condition, and reproduction. Copper appears to be related to unknown enzyme systems, involving hair pigmentation and pantothenic acid, and cellular respiration. The toxic action of molybdenum may be due to competition between molybdenum and phosphorus for deposition or interference in enzyme systems which are necessary for bone formation. The therapeutic effect of copper does not appear to be accounted for by the formation of insoluble complex compounds.

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BIOGRAPHICAL ITEMS

Leon Singer was born August 15, 1918, at Gainesville, Florida. He received the degree of Bachelor of Science in Agriculture from the University of Florida in May, 1940. He has received all of his graduate training at the University of Florida.

While in residence as a graduate student from 1940 to 1942, he was employed at the Florida Agricultural Experiment Station as a laboratory assistant in the Biochemical Laboratory of the Agronomy Department. After four years of service in the United States Army, he was discharged with the rank of captain. In November, 1946, he was employed as Assistant in Nutrition in the Animal Industry Department of the Florida Agricultural Experiment Station. He resigned this position in February, 1947, to accept a grant-in-aid fellowship from the Nutrition Foundation, Inc. While in residence, he has been a Graduate Research Assistant at the Nutrition Laboratory of the Florida Agricultural Experiment Station. He is a member of Phi Sigma honorary biological fraternity.

This dissertation was prepared under the direction of the Chairman of the candidate's Supervisory Committee and has been approved by all members of the Committee. It was submitted to the Graduate Council and was approved as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

September 3, 1949

T. M. Simpson
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